AN ECOLOGICAL STUDY OF *ULVA LACTUCA* L. AND OTHER BENTHIC ALGAE ON THE AVON-HEATHCOTE ESTUARY, CHRISTCHURCH

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CONTENTS

LIST OF FIGURES

FIGURE Page

LIST OF TABLES

LIST OF PLATES

ABSTRACT

This study comprises investigations into the ecology of the benthic algae on the Avon-Heathcote Estuary with special attention to the influence of sewage discharge on the principal species. Ulva lactuca, the most important, has been described in greatest detail.

The work was carried out in two parts, in the field and in the laboratory. In the field from May 1971 to May 1973, monthly sampling was used to relate the spatial and seasonal variation in algal abundance to relevant environmental factors, such as nutrient concentrations, exposure times, substrate availability, current velocities and grazing by Zediloma subrostrata. Algal abundance was measured from per cent cover and dry weight per area. Aerial photography was employed to map the overall distribution of the algae.

In the laboratory, the effects of phosphate and nitrate additions and variation in temperature on the growth of Ulva lactuca were studied.

The dominant alga was Ulva lactuca L. which occurred as a small attached winter plant, a larger ribbon-like spring plant, a large sheet-like detached summer plant and a smaller bullate detached plant. The latter had previously been described as Ulva laingii Chapman but appears to be the result of low salinity on aging U. lactuca thalli.

The distribution of Ulva and Enteromorpha ramulosa was restricted to the mid-tide zone due to intolerance of long exposure and low salinities. Attached algae were restricted to stable substrates while drift algae accumulated in areas with low current velocities.

Eutrophication, resulting from sewage discharge, promoted productivity in the western region of the Estuary and appeared to be the major cause of the increased productivity over the last 40 years.

Temperature emerged as the main factor determining seasonal growth patterns.

Aerial infrared photography was a successful survey tool and allowed the areas of active algae to be detected.

Plate 1.1

Oblique aerial view of the Avon-Heathcote Estuary looking towards the Bromley Oxidation Ponds with the Avon River at the top right and the Heathcote River top left.
Photo: F.E. McGregor.

Plate 1.2

The Avon-Heathcote Estuary looking towards the Port Hills from above the Avon River. Heathcote River at upper right. Photo: F.E. McGregor.

CHAPTER I

INTRODUCTION

1) GENERAL INTRODUCTION

Pollution and ecology are of considerable topical interest as man begins to appreciate the effects of his activities on his environment. These vary from complete destruction or contamination of his natural surroundings to subtle yet important changes in the ecology of the communities around him. The effects are most marked in the urban and industrial centres where the concentration of human activities brings an accumulation of waste products which often overload the natural recycling mechanisms. Man may be directly affected by pollution as in the case of toxic metal or chemical contamination, or indirectly through the changes in the ecological units of which he is a member.

The increasing pollution of the natural waters is of particular concern as water is vital to all forms of life. It is also of special importance to man as a transport medium, both for shipping and as a means of dissolving and carrying a variety of substances including waste products. The rivers can be likened to a 'blood stream' transporting dissolved and particulate matter down to the lakes and seas. This is a natural process but man, by his concentration of waste-producing processes into small areas, has frequently over-

loaded this natural system. As a result, the rivers, lakes and seas in highly populated areas have become merely extensions of the domestic and industrial sewers rather than an integral part of the wider ecosystem.

Rivers frequently open out into extensive tidal mud-flats or estuaries before finally emptying into the oceans. The broadening of the river bed and the tidal flow combine to produce a sharp drop in current velocities, resulting in increased deposition of particles suspended in the water. The flushing times often cover a number of tidal cycles so that the constant stream of dissolved and floating matter in the river water leads to accumulation within the estuaries which can therefore be more polluted than the rivers feeding into them. In many areas urban and industrial development has occurred around the shores of estuaries, often physically modifying them and adding to the pollution by direct discharge of wastes. De falco Jr (1967) described estuaries as the septic tank of the megapolis.

The inhabiting organisms vary considerably depending on the type of estuary and its location. The species can normally be grouped into five categories (Knox and Kilner, 1973).

1. The freshwater component, comprising a few salt tolerant species derived from the rivers but found only in waters of low salinity.
2. The stenohaline marine component, restricted to high salinity water near the mouth of the Estuary.
3. The euryhaline marine component, extending from the sea through the Estuary.

4. The estuarine component, which has evolved from marine forms but is now restricted to estuaries and is never found in the sea.
5. The migratory component, which includes a variety of active forms such as birds, fish and prawns which move in and out of the estuary on feeding and spawning migrations.

Estuaries are therefore not just discrete biological units but are an important part of the larger marine, freshwater and terrestrial ecosystem and their pollution or physical alteration may, therefore, have wide-ranging ecological effects.

This study is concerned with the Avon-Heathcote Estuary which lies on the east coast of the South Island of New Zealand against the northern edge of Bank's Peninsula. The general comments above are very pertinent to this Estuary which has complex and ecologically important biota, adversely affected by changes in the habitat resulting from the growth of the City of Christchurch (population 300,000) immediately to the east. These changes have included physical modification of the shore line and a reduction in water quality resulting from the discharge of domestic and industrial wastes directly into the Estuary and into the Avon and Heathcote Rivers which drain into it. The subject of this study is the effect of these changes on the communities of benthic algae.

2) DESCRIPTION OF STUDY AREA AND SOURCES OF POLLUTION

The Avon-Heathcote Estuary has an area of 8 square kilometres approximately in the shape of an equilateral

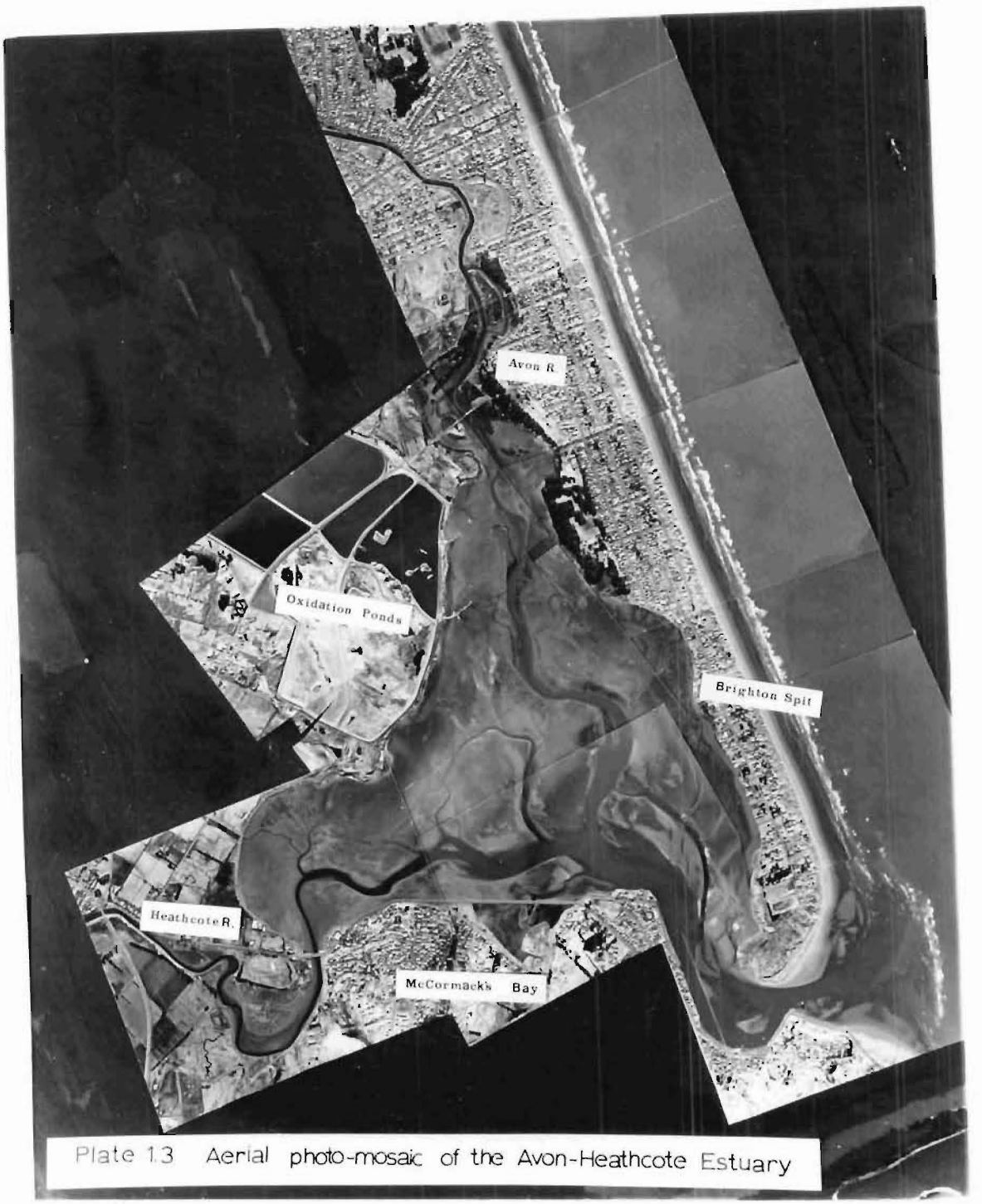


Plate 1.3 Aerial photo-mosaic of the Avon-Heathcote Estuary

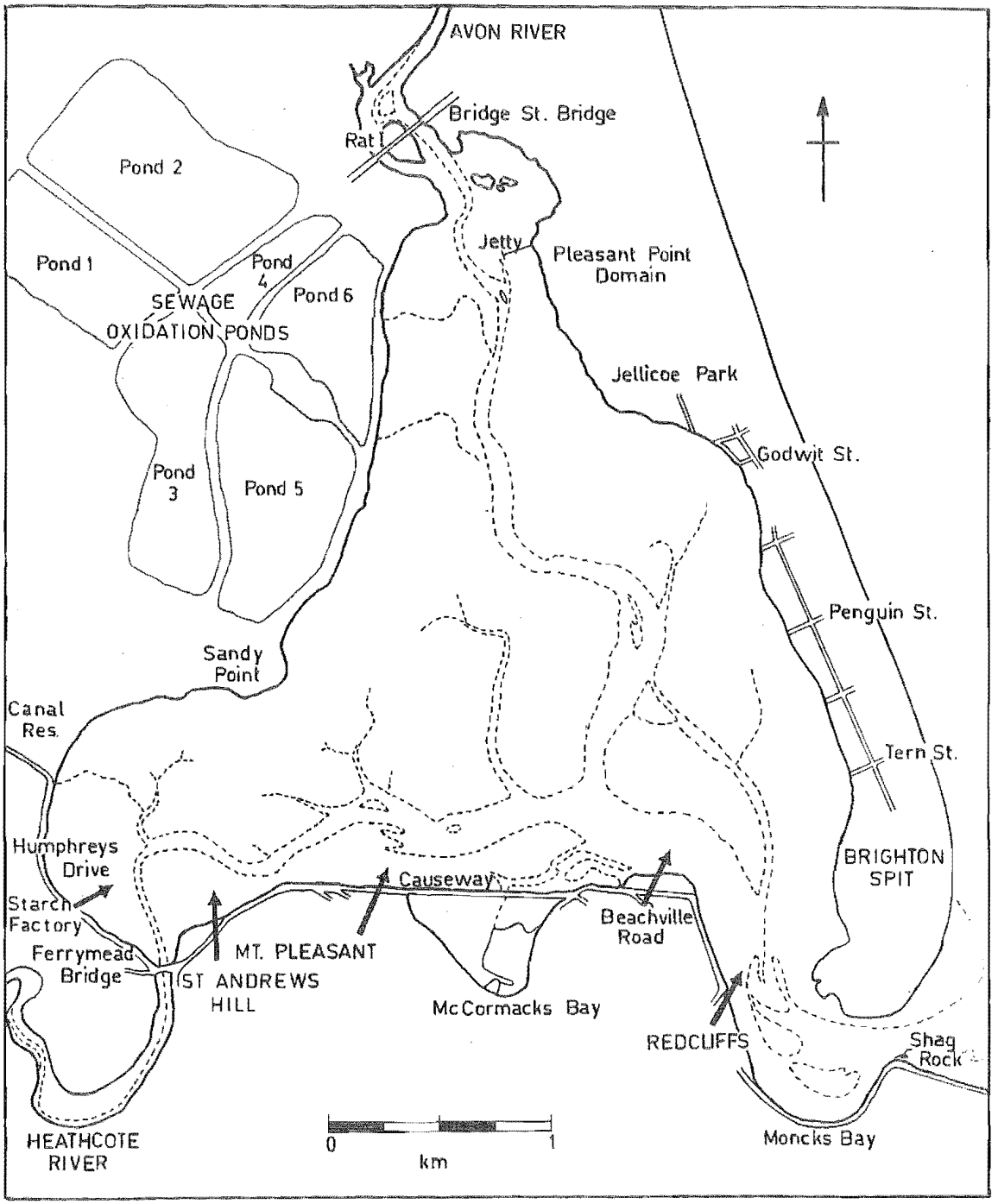


Fig. 1.1 Map of the Avon - Heathcote Estuary: arrows indicate former effluent discharges.

triangle. The southern shore is bounded by the volcanic rocks of the Port Hills, the western shore is low lying alluvial ground between the Avon and Heathcote Rivers, and the north-eastern shore is formed by the 5 kilometres long South Brighton sand spit which separates the Estuary from the Pacific Ocean (Fig. 1.1). The Heathcote River is spring fed, originating in the Port Hills with a catchment of 105 square kilometres. It flows through rural regions and the industrial section of Christchurch before entering the Estuary at the south-western corner (Fig. 1.2) where its channel continues along the southern shore to the mouth of the Estuary at the south-east. The Avon River is also spring fed, with a catchment of 84 square kilometres. It passes through the residential and commercial area of Christchurch, entering the Estuary in the northern corner from which its channel meanders across to join the Heathcote River Channel opposite Beachville Road. It has an average flow of 115 cusecs but flood volumes may reach 400 cusecs. The Heathcote River has a lower average flow of 40 cusecs but may flood to 800 cusecs (Mawson, 1972). A bar of 2 metres depth has formed at the mouth of the Estuary at the point of equilibrium between the river currents and wave action. The mixing of saline and river water extends 8 kilometres up the Avon River (Estcourt, 1962) and 11 kilometres up the Heathcote River (Cameron, 1968).

Most of the shore line has been affected by roading and housing development with retaining walls and coarse rubble being used to prevent erosion in the central areas of Brighton Spit and along the entire southern shore. A causeway on the southern shore cuts McCormack's Bay off from the rest of the Estuary apart from flow through two culverts. The main flow

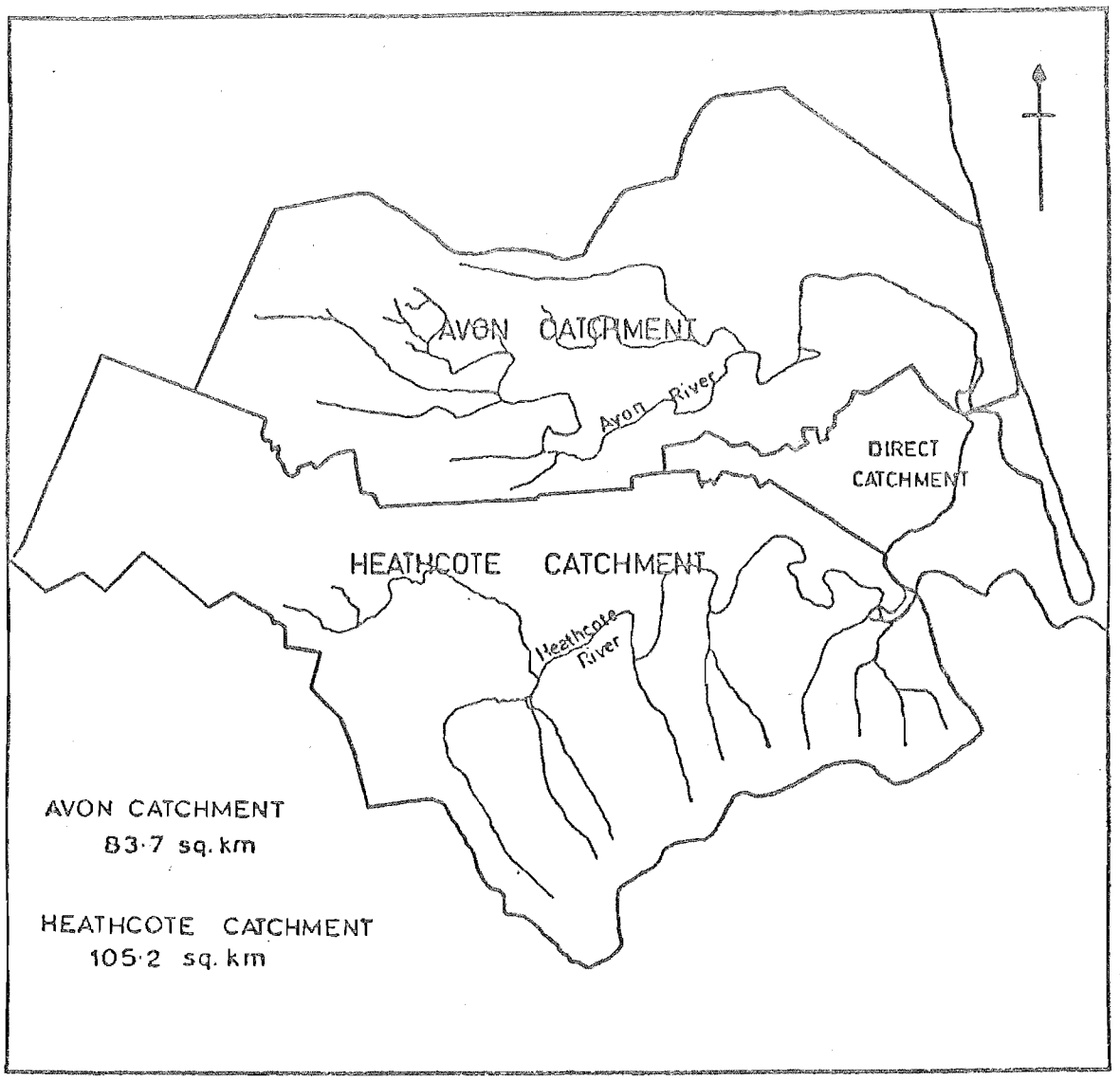


Fig. 1.2: Catchments of the Avon and Heathcote Rivers.

occurs through the central culvert which is placed to the west of a bank running north - south across McCormack's Bay. This bank retains water in the eastern portion while the western portion drains completely, but is exposed for a shorter period at low tide than is the main body of the Estuary. Reclamation by the Christchurch City Council is proceeding and will further decrease the area of McCormack's Bay.

As noted above, the lower reaches of the Avon River pass through the residential and commercial areas of Christchurch while the Heathcote River passes through the more industrial sector. Both receive storm water and some effluent discharge. The major industrial effluents which in the past were discharged directly into the Heathcote River have since 1973 been piped to the Bromley Oxidation Ponds. The notable exception is the Christchurch Gas Company effluent which is still discharged into the Heathcote River as the effluent would need pretreatment before being permitted to pass through the sewage treatment system. The Estuary also receives some storm water drainage, the most important being from Canal reserve (Fig. 1.1). Until recently, untreated sewage was also discharged directly into the Estuary but extensions to the sewer systems now pump the sewage from surrounding areas to the Bromley Oxidation Ponds. The recent changes are outlined below (see Fig. 1.1 for locations).

Table 1.1: Recent changes in effluent discharge

Effluent Origin	Date of cessation of discharge into the Estuary
St Andrew's Hill	December 1965
Mt Pleasant	March 1966
Starch Factory	April 1968
Redcliffs	January 1970
Beachville Rd	March 1970

The effluents from the Bromley Oxidation Ponds discharge at two points on the western shore, the largest volume coming from the northern pipe. The volume of discharge

has increased steadily since the present oxidation ponds replaced the sewage farm in 1961. Changes in average daily flows since 1929 are shown below.

Table 1.2 Changes in daily flow from the Bromley Oxidation Ponds (after Knox and Kilner, 1973)

Year	Volume Million gal./day
1929	8.1
1958	16.1
1963	17.0
1965	22.0
1968	23.0
1971	25.4
1972	28.4
1980 (estimate)	35.0

General Biology of the Avon-Heathcote Estuary

The Estuary contains an abundance and variety of organisms, predominantly euryhaline marine species. There are small numbers of exclusively estuarine, stenohaline marine, and salt tolerant freshwater species. A total of 130 species of invertebrates has been recorded (Knox and Kilner, 1973) with a further group unidentified, particularly protozoa. The dominants are the benthic molluscs such as Chione stutchburyi, Amphidesma australe australe, Zediloma subrostrata and Amphibola crenata.

There is also a large bird population of which 28 species are recorded as common with the South Island pied Oyster Catcher (Haematopus ostralegus finschi) particularly abundant, feeding on the Chione beds.

A total of 26 species of fish have been recorded, some

as permanent residents, others are seasonally present. The main commercial species is the sand flounder Rhombosolea plebeia. The Estuary is also the main nursery for the off shore flounder fishery (Mundy, 1968).

The flora includes 60 species of angiosperms around the margin of the Estuary, the most common being Juncus maritimus, Leptocarpus simplex, Scirpus americanus, S. caldwelli, S. lacustris, Plagianthus divaricatus and Spartina townsendii.

The microscopic organisms are not well known but records include two species of Euglena, one Cyanophyta (Oscillatoria), eleven Chrysophyta (diatoms) and one Fungus (Sphaerotilus).

The most noticeable component of the flora is the benthic algae which include species from the Chlorophyta and Rhodophyta.

This brief survey indicates that the Estuary forms the habitat (in part or in whole) of over 260 macroscopic species. It is therefore a unit of considerable biological importance in its own right and as part of a wider ecological pattern. Physical alteration of the Estuary and the contamination of its waters by increasing discharges of domestic and industrial wastes is therefore of great concern to local biologists.

The Estuary also plays an important part in the lives of the people of Christchurch. Its proximity to the city has made it a popular area for boating, fishing and nature study, and it is the immediate environment for the suburbs around its shore. It also plays an important role in the storm water drainage of the city. The reduction in water quality and biological changes which have occurred in recent years detract from the recreational potential and aesthetic qualities of the area.

Concern for the biological state of the Estuary has prompted studies aimed at assessing the impact of pollution on the biota. These studies have included a number of surveys by students of the Zoology Department, University of Canterbury, with the financial support of the Christchurch Drinage Board. These studies are reviewed in more detail in Chapter II. The biological change on the Estuary which has caused the most widespread concern is the development of very large quantities of green seaweeds. These now dominate the Estuary at the expense of the rest of the community. They also inconvenience fishermen and boaters. In some summers the accumulation of the seaweeds above the high tide mark causes a fouling of the air and has been blamed for paint damage on nearby buildings.

Very large increases in the mass of seaweeds have been associated with increasing eutrophication of the waters resulting from sewage pollution.

Proposals by the Drainage Board for a barrage at the mouth of the Estuary and major channel dredging for flood control would involve very extensive modification of the area. To evaluate the possible effects of these proposals and to continue work on the effect of pollution, the Drainage Board has supported a more intensive biological study of the Estuary under the supervision of Professor Knox of the Zoology Department, University of Canterbury. There has been close co-operation between Professor Knox's programme and my own study.

3) NATURE AND SCOPE OF STUDY

The ultimate aim of this study was to evaluate the

association between eutrophic water and the excessive growth of benthic algae on the Estuary. As nutrients are only one of many factors affecting the algae, it is necessary to have a knowledge of the general ecology of the species involved before the effects of nutrients can be assessed. The problem was approached in two different ways. The first involved a two year field study aimed at determining the pattern of spatial and seasonal variation in the algal populations and monitoring environmental variables likely to affect this pattern. The second method was a laboratory study of the effect of temperature and nitrogen and phosphorus levels in the water on growth rate. The laboratory study was necessary to separate the effect of these parameters from the rest of the factors acting on the algae in the field.

CHAPTER II

LITERATURE REVIEW

1) REVIEW OF PREVIOUS ALGAL STUDIES ON THE AVON-HEATHCOTE ESTUARY

The first biological study on the Estuary was an investigation of the littoral ecology by Thompson (1929). Although the algae were not covered in detail, it provides very useful information as to the biological state of the Estuary prior to its being seriously polluted. This study was followed by four summer surveys between 1951 and 1965 aimed at assessing the effect of pollution on the biota. The first study to concentrate on the algae and provide seasonal data was carried out by Cameron 1968/69.

(a) Thompson (1929)

Thompson noted that "the Estuary shows remarkably little flora". The vascular plants mentioned were sparse stunted patches of Zostera on the sandy regions and a marginal salt reed formation of Leptocarpus simplex and Juncus maritimus. The algae mentioned were Syphonocladis cladophera which formed thick mats on the mud, Ulva sp. and Scytothamnus australis, both growing on stones and shells in sheltered rock pools and on the mud banks.

In the same year, specimens of Ulva lactuca and Gracilaria sp. were collected by Laing (Laing Herbarium,

University of Canterbury, Christchurch).

(b) Bruce 1950/51 (Reported 1953)

This was the first in a series of summer surveys supported by the Christchurch Drainage Board to assess the biological impact of pollution on the Estuary. Bruce found very little Zostera but recorded considerable quantities of Ulva which, she noted, had been abundant since 1946.

The main concentrations of Ulva were in the South and West on the mudflats adjacent to the Heathcote River Channel, particularly in areas which did not drain completely at low tide (see appendix 1, Fig. I). Most of the Ulva in this area was unattached, lying in drifts of densities up to 12.6 lb wet weight per square metre. Wet weight was determined by squeezing to remove superfluous water and weighing on a spring balance. Attached Ulva occurred in lower concentrations over most of the Estuary.

Bruce also recorded Scytothamnus australis but later changed this to Gracilaria after the material was identified by V.J. Chapman. There is nothing to prove that this is the same alga listed by Thompson (1929) as S. australis.

The third macroscopic alga recorded by Bruce was Enteromorpha sp. which occurred in the low salinity area near the Bridge St. bridge.

A band of yellow-green slime occurred along the high tide mark on the Western shore. This consisted of numerous diatoms, Euglena limosa and patches of Oscillatoria. This slime was first recorded by Linzey (1944).

Bruce also examined the nitrogen and sulphur content in the thallus of the algae. Her results will be discussed in

relation to levels found by Cameron and in overseas studies in Chapter VIII.

(c) Williams 1958/59 (Reported 1960)

Williams recorded a distribution of Ulva similar to that in 1950/51 but was equalled in abundance by Enteromorpha sp. (see appendix 1, Fig. II). Ulva and Enteromorpha combined reached densities up to 32 lb wet weight per square metre. Gracilaria was common, but Zostera was very scarce. The lack Zostera was attributed to the increase in water pollution. Durvillea and Macrocystis fragments were found in the drift, presumably originating from outside the Estuary. Ulva fragments were also seen to enter the Estuary on the flood tide.

Oscillatoria was found in the vicinity of the Bromley Oxidation Pond effluent. Although associated with highly organic muds, it did not grow in the dark on organic substrates, indicating an inability to carry out heterotrophic nutrition. Williams also recorded Euglena limosa and a second species E. salina from the Heathcote River.

(d) Rosenberg 1962/63 (Reported 1963)

Ulva and Enteromorpha were present in equal amounts, with Ulva occurring further up the shore. The algae were mostly attached and at much lower density than that reported by Bruce and Williams (see appendix 1, Fig. III). Gracilaria was sparse and Porphyra sp. occurred in the channels near Beachville Road. Chaetomorpha linum was recorded for the first time as floating masses of filaments in the imponded region of McCormack's Bay.

(e) Webb 1964/65 (Reported 1965)

Webb reported a resurgence of Ulva and Enteromorpha, and identified the species as U. lactuca and E. intestinalis. Also mentioned were Oscillatoria (near oxidation ponds), Spirogyra, Stigeoclonium, Ulothrix, Euglena limosa and E. salina. The freshwater species were presumably from areas near the river mouths (see appendix 1, Fig. IV for distribution).

(f) Cameron 1969/70 (Reported Knox and Kilner, 1973)

This was the first survey to include all four seasons. Samples were taken in January, April, July and October 1969 and January 1970. The algae were harvested from quadrats and dried at 110^{o}C before being weighed. Species recorded were Ulva lactuca, Enteromorpha ramulosa, E. intestinalis and Gracilaria sp. Enteromorpha intestinalis was found in McCormack's Bay in the same region from which Rosenberg recorded Chaetomorpha linum and near the Bridge St. bridge forming large floating drifts. The survey concentrated on Ulva lactuca and Enteromorpha ramulosa which made up the bulk of the algae.

In January 1969 the main concentrations of Ulva and Enteromorpha occurred in the western half of the Estuary particularly on the mud flats adjacent to the Heathcote River channel and south of the Bromley Oxidation Pond outlet (see appendix 1, Fig. V - Fig. IX). The samples were 80% Enteromorpha and 20% Ulva. In April the area covered by the algae had decreased but retained the same distribution pattern. However, the ratio of Enteromorpha to Ulva was the reverse of the January figures being 80% Ulva and 20% Enteromorpha. The

area covered by the algae remained stable until October 1969 when it decreased. It increased again in January 1970 to an area similar to that covered in 1969, but Ulva remained dominant over Enteromorpha. The percentage of the Estuary covered by the algae and the mean weight of algae did not always correspond. The total dry weight for January 1970 was lower than for January 1969 whereas the area covered was slightly larger. This discrepancy may be due to the drifts of algae being of varying thickness so that the 100% cover category may include a variety of densities.

Summary of algal changes on the Estuary prior to the present study

Due to the small number of samples, the lack of seasonal data and the use of wet weight in the earlier surveys it is not possible to make precise comparisons between them and Cameron's work, however, some general trends are discernible. The most noticeable change has been the increase in abundance of Ulva since 1929, and more recently the increase in Enteromorpha. Ulva has been abundant since 1946 but has shown considerable year to year variation. In the period of sparse Ulva recorded by Rosenberg 1962/63 most of the plants were attached.

Enteromorpha rose very quickly to be the dominant species in the 1968/69 summer but was not dominant in 1969/70. Cameron noted the association between the Enteromorpha bloom and unusually high atmospheric temperatures in the preceding month. The Enteromorpha abundance was markedly reduced in both winters included in Cameron's study. The typical pattern of distribution has been a concentration of drift algae on the mud flats adjacent to the Heathcote River Channel and near the

Oxidation Ponds, particularly in depressions and pools on the mud flats. The attached plants have had a more general distribution and lower density.

Gracilaria has been consistently recorded as fairly common but has always been dominated by Ulva and latterly by Enteromorpha. The only other macroscopic algae listed were Enteromorpha intestinalis, Porphyra and Chaetomorpha linum but they have never been widespread.

Apart from isolated records of Ulothrix, Spirogyra and Stigeoclonium by Webb (1965), the major microscopic algae occur in the patch of surface scum stretching from the Avon to the Heathcote River, being especially dense near the oxidation ponds. They consist of numerous species of diatoms, Oscillatoria and two species of Euglena. The distribution of Euglena is covered more fully by Steffensen (1974) (see appendix II). The Euglena has the potential for heterotrophic nutrition which may explain its association with highly organic muds. Williams (1960) found no evidence for heterotrophy in the Oscillatoria.

Zostera, the only vascular plant recorded in the central region, has generally been very sparse. However, the present study has indicated that it is increasing in abundance, particularly near the Avon River channel where it forms raised patches up to 10 metres in diameter. The main change in the marginal vegetation has been the increase in Spartina townsendii which was introduced near the Bridge St. bridge some time prior to 1968 to consolidate the banks (Bascand, 1968). It has spread over large areas of the northern and western shores causing some concern because of its tendency to accumulate silt around the base of clumps and thus may

decrease the intertidal area of the Estuary. Much of the salt reed formation mentioned by Thompson (1929) has been eliminated by reclamation and the dumping of spoil around the shoreline.

The change from a sparse flora as described by Thompson (1929) to the present super-abundance of Ulva and Enteromorpha and the development of the Euglena/Oscillatoria/diatom scum have been linked with the increased pollution, particularly the eutrophication resulting from sewage disposal (Bruce, 1953; Williams, 1960). Although no quantitative data are available, similar biological changes appear to have taken place in a number of estuaries in other parts of New Zealand which have also been subjected to increased pollution. Ulva and Enteromorpha growths are causing problems in the Moutere Inlet and Nelson Haven in the Nelson district, Blake Town Lagoon, Greymouth and in the Porirua estuary, Wellington which are all subject to sewage pollution. Similar patterns of change have been recorded in polluted waters overseas in Europe, India, North America and the Carribean.

2) REVEW OF OVERSEAS STUDIES

In a number of overseas studies abnormally luxuriant growth of Ulva and Enteromorpha, similar to that in New Zealand estuaries, has also been recorded. These records, all from waters polluted by sewage effluent, are listed below under geographical headings.

(a) Ulva

North America

Sawyer (1965), Rhyne and Hommersand (1970), Waite and

Mitchell (1972a).

Europe

Cotton (1910), Letts and Richards (1911), Sundene (1953), Grenager (1953), Den Hartog (1959), Gamulin et al (1967), Ariales and Morales (1969), Golubic (1970), Edwards (1972).

Carribean

McNulty (1961).

India

Subbaramaiah and Parekh (1967).

(b) Enteromorpha

Europe

Waern (1952), Sundene (1953), Grenager (1953), Arias and Morales (1967), Edwards (1972), Perkins and Abbott (1972).

North America

Phinney and Lyford (1968), Waite and Mitchell (1972a).

A number of other species have also been noted in large quantities in association with pollution from sewage effluent. These are listed below in order of importance.

Prasiola stipitata: Sundene (1953), Taylor (1937), Den Hartog (1958), Lewin (1955), Edwards (1972)

Rosenvingiella polyrhiza: Den Hartog (1959), Edwards (1972)

Bangia fuscopurpurea: Grenager (1953), Waern (1952)

Capsosiphon fulvescens: Waern (1952), Edwards (1972)

Blidingia minima: Sundene (1953), Edwards (1972)

Porphyra umbilicus: Grenager (1953)

Fucus vesiculosus: Haahtela (1970)

Gracilaria blodgetti: McNulty (1961)

Although these algae also occur in areas which are not polluted, the very luxuriant growth and large thallus size is normally restricted to polluted areas. Muenschler (1915) and Setchell and Gardner (1926) described dense beds of Ulva in quiet backwater bays not noticeably polluted, but these appear to be the result of fragments detached from the sparser attached populations in the surrounding area, rather than of high productivity in the beds. With these two exceptions, extensive areas of luxuriant benthic algae such as Ulva and Enteromorpha on estuaries are invariably associated with sewage pollution.

Blooms of marine and freshwater planktonic algae in association with water pollution have also been widely reported and have usually been attributed to an enriched nutrient supply. Ketchum (1969) points out that excessive eutrophication of estuaries and coastal waters leads to blooms of obnoxious and non-endemic species, to the detriment of desirable species. The association between nutrient enrichment and algal blooms is generally accepted. The current position is summarised by Clesceri et al (1973): "No question has yet been raised as to the efficacy of nutrients for sustaining and being requisite for the growth of algae. The only query yet to be answered is what concentrations of specific nutrients are required for sustained growth of a nuisance algal bloom in a particular situation".

Organic and inorganic micronutrients have received relatively little attention, although Provasoli (1958) has demonstrated the importance of micronutrients in the growth processes of Ulva lactuca. Knox and Kilner (1973), however,

do not regard micronutrients as likely to limit growth in eutrophic estuaries.

Although carbon as a limiting factor has received some attention by Kuentzel (1969, 1970), King (1970, 1972), Kerr et al (1972) and Wetzel (1972), the abundance of dissolved organic matter present in polluted areas precludes it from limiting growth in these areas (Parker, 1967; Shapiro, 1970; Vallentyne, 1972).

Most attention has therefore been focused on the macronutrients, phosphorus and nitrogen which are regarded as the major factors limiting growth in aquatic algae by Ketchem et al (1958), Lund (1965), Fruh (1967), Stewart and Rolich (1967) and Steel (1972). Phosphorus and nitrogen nutrition will be treated in greater detail in Chapter VIII.

Moore (1971) posed the following questions: "is there an ecological pattern correlated spatially with areas of pollution and how will this pattern be modified by future extension or intensification of pollution"? It seems to be widely held that the pattern characteristic for polluted areas is a decreased species diversity but with a greater abundance of the remaining species. It is not entirely clear whether the "clean" water species are eliminated by toxic elements in the pollutants or whether they are excluded by the vigorous growth of the "pollution" species. In cases of industrial pollution, the extension of the tolerant species may be more passive than in cases of pollution from domestic sewage where the high nutrient levels may give a competitive advantage to the "eutrophic" species. The luxuriant growth of algae such as Ulva lactuca in sewage polluted waters suggests that growth is stimulated under these conditions and that

their abundance is not simply the result of extension into previously occupied niches.

An indication of possible future developments can be seen in modification of the existing spatial patterns from unpolluted to progressively more polluted areas. Gamulin et al (1967) examined changes in community structure in polluted waters near Naples and found that species diversity decreased towards the source of sewage pollution until the community was reduced to two species of algae (Ulva lactuca and Hypnea musciformis) and one species of animal (Aphysis fasciata). The algae extended as a dense bed right up to the death point where the whole community abruptly broke down. The authors noted that decreasing diversity is typical of communities subject to extreme environmental conditions and that similar trends occur in areas of sharp gradients in temperature, salinity and exposure time. They also regarded the unstability of the community as predictable from the low species diversity.

CHAPTER III

MATERIALS AND METHODS FOR FIELD STUDY

A number of difficulties were met in designing a sampling programme which would allow to adequately estimate the standing crop of algae on the Estuary. A discussion of these difficulties is presented to justify the choice of methods used.

1) GENERAL DISCUSSION OF METHODS

The nature of the study area and the type of algae presented a number of practical difficulties. Firstly, the large area of the Estuary, the difficulty of access to some localities due to patches of very soft mud and to the river channels, and the enforced restriction of sampling to low tide combined to limit the number and distribution of sites. A second set of sampling difficulties centred on the need to deal adequately with both attached and drifting algae. Although the attached algae vary in density over the Estuary as a whole, it can be subdivided into areas within which the attached algae are reasonably homogenecusly distributed. In many respects these areas resemble a sparse grassland for which a number of satisfactory techniques for measuring biomass or cover have been developed, as discussed by Walker (1970).

The drift algae present a more unusual problem both in designing an adequate sampling programme and in interpretation of results. Since at low tide the detached algae lie in very clumped patches, random distribution of sample points over the whole Estuary would be either inadequate or unrealistic. As the sheets of drift algae may be several layers thick, their density cannot be estimated by means of percentage cover as is done for the attached algae.

Some of the difficulties were mentioned by Cameron (1968) who also had difficulty in sampling the algae. Cameron selected 37 sites from where she harvested from 0.1 metre square quadrats. She also mapped the percentage cover of the Estuary as a whole, using four categories: no cover, 1-25%, 25-75% and 75-100%. She regarded percent cover as a more reliable estimate of overall abundance due to the possible bias of the harvest quadrats to the denser patches of algae. This disregards the very wide range of density possible in the 75-100% cover category.

From the above discussion it appeared dry weight per area would be superior to cover as it could give comparable results for both attached and drift algae. However, harvesting requires more time in the field per sample than percent cover estimates do. The period available for field work at each sample area and the frequency of visits are limited by the tides. This and the time required to separate the species from each other and from the shells and snails usually associated with the algae restrict the number of samples which can be processed.

A further problem in designing a suitable field study plan was the uncertainty surrounding the taxonomic status of

the algae on the Estuary. Although this thesis is primarily ecological in nature, it is important that the algae be clearly separated into species. The taxonomic investigation was therefore designed to furnish descriptions in sufficient depth to separate the algae into species with reasonable confidence and to define the main morphological stages of their life histories. This proved more difficult than had been expected, particularly for the Ulva specimens. The existing keys and descriptions were unsatisfactory as it was not clear whether the specimens on the Estuary belonged to several different species or were all different forms of a single species. As this had a bearing on the ecological interpretations, the Ulva specimens have been studied in greater detail than those of the rest of the community which presented fewer problems in identification.

In a study of this type it is desirable to examine as many environmental variables as possible if the causes of the spatial and seasonal variation in the algae are to be sought. By co-ordination of this study with the programme being carried out on the Estuary by the Zoology Department this aspect has been extended further than would otherwise have been possible in a thesis of this type. Even so, it is necessary to select those variables which have the greatest ecological impact on the algae.

In order to make the best use of the limited number of samples and sample sites, which it was feasible to use, it was necessary to identify the ecologically most important areas on the Estuary, to clarify the taxonomic status of the algae and to evaluate the relative importance of the wide range of environmental variables which could affect the algae. A

preliminary field study was carried out in 1971, using a rapid percent cover estimate as the criterion for abundance. Although this technique did not provide exact quantitative results in areas with overlapping layers of drift algae, it was possible to take a much larger number of samples and to cover a much wider area than with a dry weight method. These results were used to plan the more intensive and quantitative study carried out in 1972 and 1973.

2) METHODS FOR INITIAL SURVEYS MAY 1971 TO JANUARY 1972

(a) Taxonomic survey

In order to arrive at a species list, monthly collections of representatives of the algae present were made each month and their morphology and anatomy was described. The seasonal changes in thallus size and morphology was followed in the field in ten permanent metre square quadrats marked 1 to 10 on Fig. 3.1. The size of each thallus was determined from the photographs by a planimeter. Initially the photographs were taken with a 5 x 4 inch format Linhoff camera mounted on a tripod to one side of the quadrat with the quadrat brought into square with the rising front adjustment on the camera. This system was time consuming and awkward to use on the soft mud of the Estuary and was replaced by a 35 mm camera mounted on an aluminium frame attached to the four corners of a metre square quadrat. The camera was fixed on a platform 1.5 metres above the centre of the quadrat (Plate 3.1). This frame was light to carry and required a minimum of adjustment between photographs. Colour negative film was used giving a choice of colour or black and white prints to be

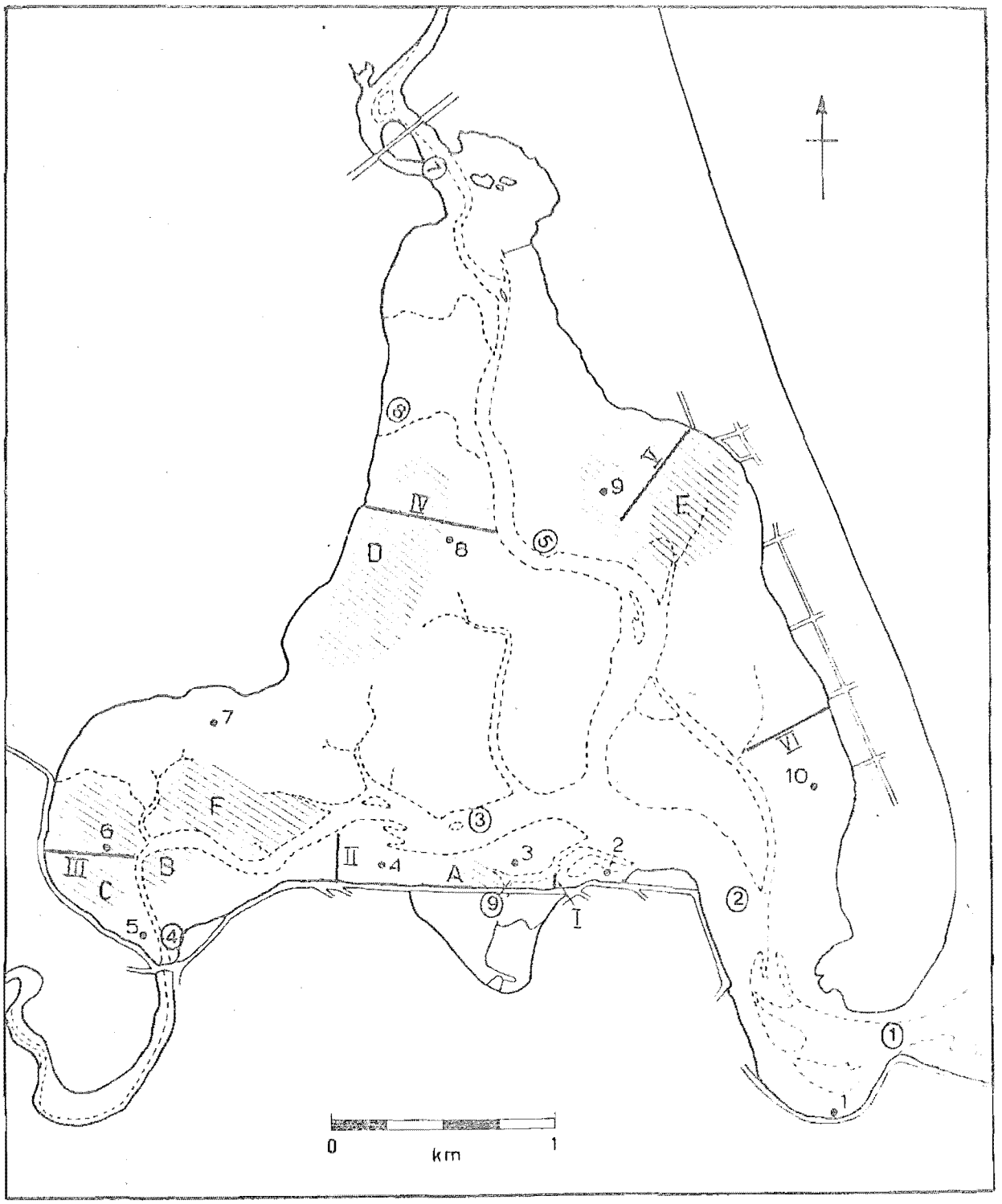


Fig. 3.1 Position of algal sample areas A-F, transects I-VI, permanent quadrats 1-10 and channel water sample stations ① - ⑨.

Plate 3.1

Optical point sampler showing detail of the viewing system.

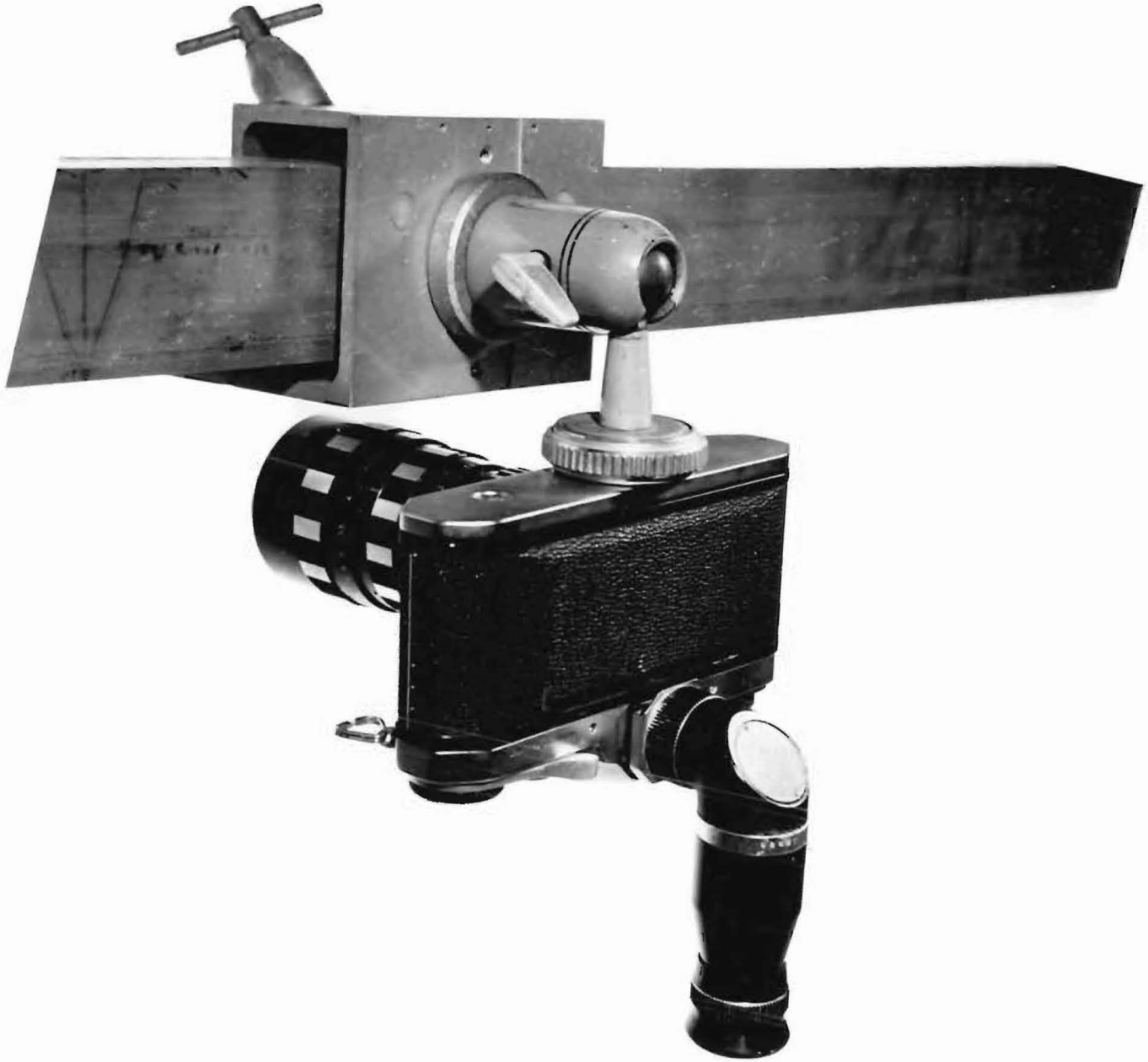
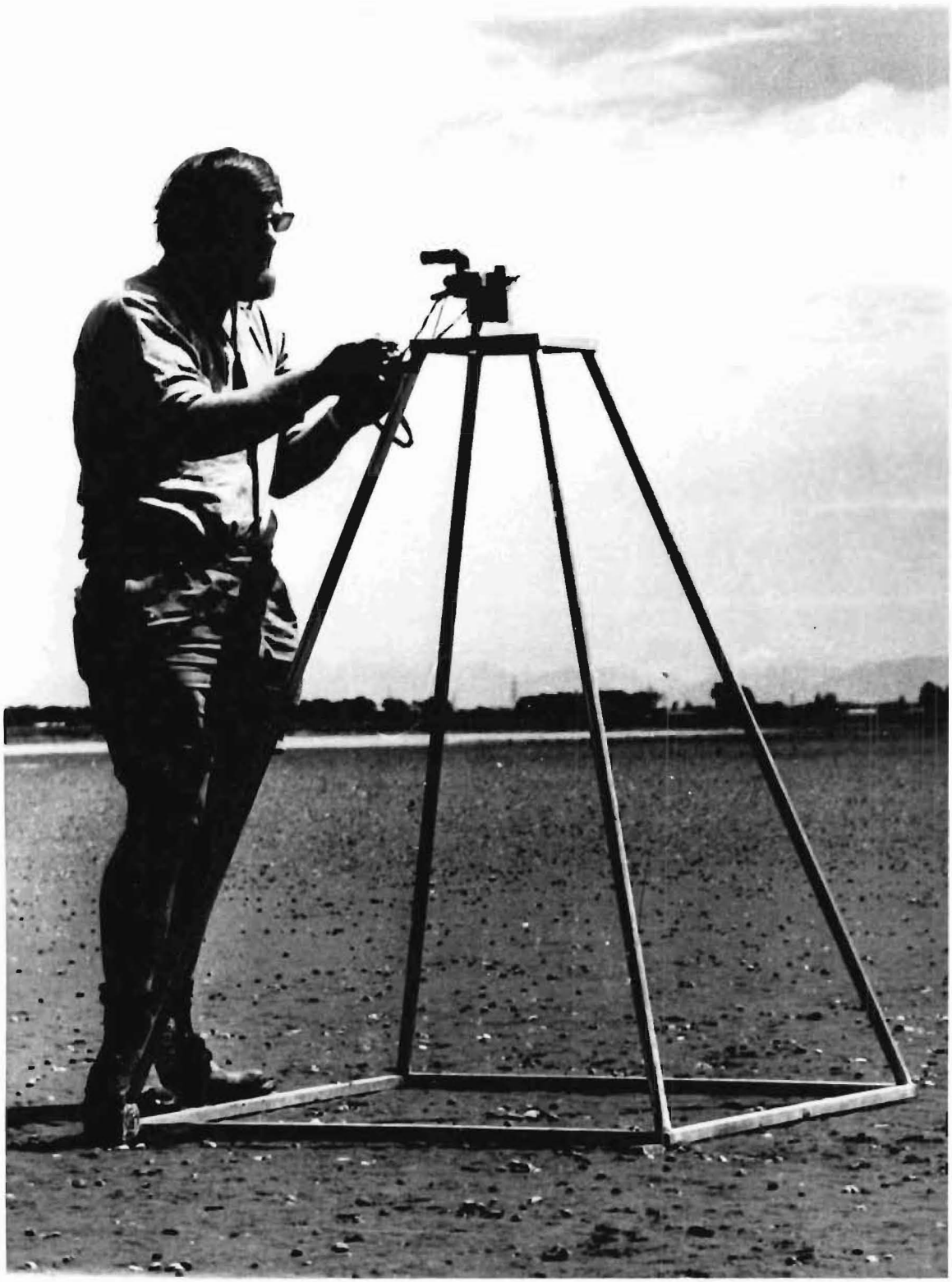


Plate 3.2

Frame used to obtain vertical photographs of the quadrats.



produced, depending on the importance of each photograph. The resolution of the 35 mm camera did not match that of the 5 x 4 inch format, and the legs of the frame often cast shadows over the quadrat. This small loss in photographic quality was more than compensated for by the reduction of time spent at each sampling site.

(b) Quantitative methods

For the initial surveys of standing crop levels, per cent cover was used as a measure of density as it made possible a much greater number of samples over a wider area than dry weight determinations. The samples were taken along transects at positions I to VI on Fig. 3.1. All transects ran from the high-tide mark to the river channel and placed so as to include the main algal communities. Samples were also taken at random in the areas between the transects. Per cent cover was estimated using an optical point sampler modified after Morrison and Varrautau (1970). It consists of a 2 metre graduated aluminium bar supported at each end by two adjustable legs. A camera fitted with a 135 mm lens, waist level viewer, and a hairline cross on the screen, is mounted on a movable carriage on the bar (Plate 3.2). Sightings could be made through the camera from the standing position and the presence or absence of algae under the cross noted. The bar was set at right angles across the transect and 10 sightings were made at 20 centimetre intervals along the bar. This procedure was repeated at 5 metre intervals along the transects.

The per cent cover data from the optical point sampler and the photographs of the quadrats were combined to produce

maps of per cent cover over the Estuary as a whole for each month.

3) METHODS FOR SECOND PHASE OF SAMPLING PROGRAMME - JANUARY 1972 TO MAY 1973

The results of the preliminary study clearly indicated that the drift algae must be taken into account when assessing total standing crop. As the drift was not adequately sampled by per cent cover measurements, the main emphasis was switched to dry weight determination. The algae were harvested from quarter metre square quadrats, sorted into species and dried to constant weight at 110°C.

Distribution of sampling points (Fig. 3.1)

From the preliminary study it appeared that the main areas of attached plants occurred in the south-west region of the Estuary in association with a number of shell beds. Areas Aa and Ba were selected as representative of this region. Area E was chosen as representative of the northern region of the Estuary characterised by attached plants. The areas were marked out and five samples taken at random within each. Within the drift patches immediately adjacent to areas Aa and Ba samples were also taken at random, and the drift at areas C and D was sampled in the same way. The areas covered are shown on Fig. 3.1. Per cent cover determinations were continued from photographs of the quadrats in the areas of attached plants.

Since November 1971 the overall distribution of the algae was determined from aerial photomosaics. As this method is distinct from the other field methods used and applies to

more than one aspect of the study, the method and its results are discussed in a separate chapter.

4) MONITORING OF ENVIRONMENTAL VARIABLES

(a) Water chemistry

Members of the Zoology Department have carried out a programme of water analysis on the Estuary determining salinity, phosphorus as PO_4 and nitrogen as NO_3. From January 1970 to September 1971 monthly sampling took place in the channels at stations ① to ⑨ Fig. 3.1. One surface and one mid-water sample was taken at each station at low tide. These stations unfortunately are not closely associated with the algal sampling sites. From May 1972 the monthly water samples were taken over the algal sampling sites at 30 minute intervals throughout the tidal cycle and analysed as previously. Phosphorus levels were determined using the molybdate blue method (Strickland and Parsons, 1968). Nitrate was measured by the Brucine method (Jenkins and Medsker, 1964). Data on the nutrient levels in the sewage effluent and the river water have been provided by members of the Zoology Department.

(b) Dispersal of sewage effluent

The dispersal pattern of the sewage discharged from the Bromley Oxidation Ponds has been followed from aerial photographs taken at 90 minute intervals over neap and spring tide cycles. In addition, the dispersal of dyes released into the Estuary has been observed from the air and from the ground. 6 lb of rhodamine dye was released 10 metres out from the northern discharge pipe of the oxidations, and 3 lb of fluoresceine dye a further 200 metres off shore in the channel

of the Avon River. The dye release took place just as the tide began to ebb, and their progress was recorded at regular intervals by aerial photography and by observations from a small boat. Directions were given to the boat from the aircraft via a radio link up to keep the boat at the leading edge of the dye patches.

(c) Current patterns and velocities

The current patterns on the Estuary have been described by Webb (1972). This work was supplemented in this study by the surveys of sewage and dye dispersal as described above.

Data on current velocities have been made available from the Wallingford Hydrological Report to the Christchurch Drainage Board.

(d) Exposure time

The overall exposure/submergence pattern for the Estuary was determined from the aerial surveys as described above and supplemented by more detailed ground observations at each sample site.

(e) Grazing pressure

The herbivorous epibenthic snail Zediloma subrostrata is common on the Estuary, and is often observed feeding on the algae. The Zediloma present in the harvested quadrats were counted and weighed. Data on their distribution in 1969 were provided by Kilner (1969).

(f) Substrata

The nature of the substrata and the availability of suitable algal attachment sites were noted for each sampling site. Data on the particle size and nutrient content of the

sediments were provided by members of the Zoology Department.

(g) Meteorological data

Meteorological records of air and grass temperature were made available from the Bromley Sewage Treatment Station. Insolation and hours of sunshine records were obtained from Christchurch International Airport as this was the nearest recording station.

(h) Experimental field work

An attempt was made to assess the relative effects of grazing and of removal of plants by the currents on the standing crop in different sample areas. Nine metre square quadrats were enclosed by a steel frame covered by fine netting, standing 40 centimetres high. The frames were partitioned in half by a netting barrier, secured to pegs and set into the mud to prevent movement of algae in or out. All the Zediloma were removed from one half of the frame while the remaining half was left untouched. The frames were placed in areas where the algae were evenly distributed so that similar levels of algae occurred in each half. The enclosed quadrats were photographed each month together with an immediately adjacent open quadrat and the algae harvested after three months. In this way the effect of enclosure with and without herbivors was assessed.

CHAPTER IV

AERIAL PHOTOGRAPHIC SURVEYS

1) MATERIALS AND METHODS

(a) Introduction

The aim was to develop a simple aerial survey technique to improve on the speed and accuracy of the ground surveys of the algae, and to up-date the existing maps of the channels and shoreline of the Estuary. The technique was developed in association with F.E. McGregor, photographer, Department of Botany, University of Canterbury.

Aerial photography has been successfully applied to terrestrial ecology, particularly forestry and agriculture (Anson, 1966; Billings and Morris, 1951; Ciesala et al, 1967; Haack, 1962; Knipling, 1969). More recently its use has extended to studies of Marshland (Anderson and Wobber, 1973; Olson, 1959; Seher and Tueller, 1973; Reimold et al, 1973; and Wallentinus and Tueller, 1973) and of shallow water benthos (Cameron, 1950; Kelly, 1968, 1969; and Kumpf and Randall, 1961). These surveys involved equipment and techniques outside the scope of my own study. Harris and Haney (1973) demonstrated the feasibility of low budget oblique infrared photography from a slightly modified light plane. The present project extends this type of technique to vertical photography to allow accurate maps to be produced.

Plate 4.1

Paired 35mm cameras as mounted in the aircraft.

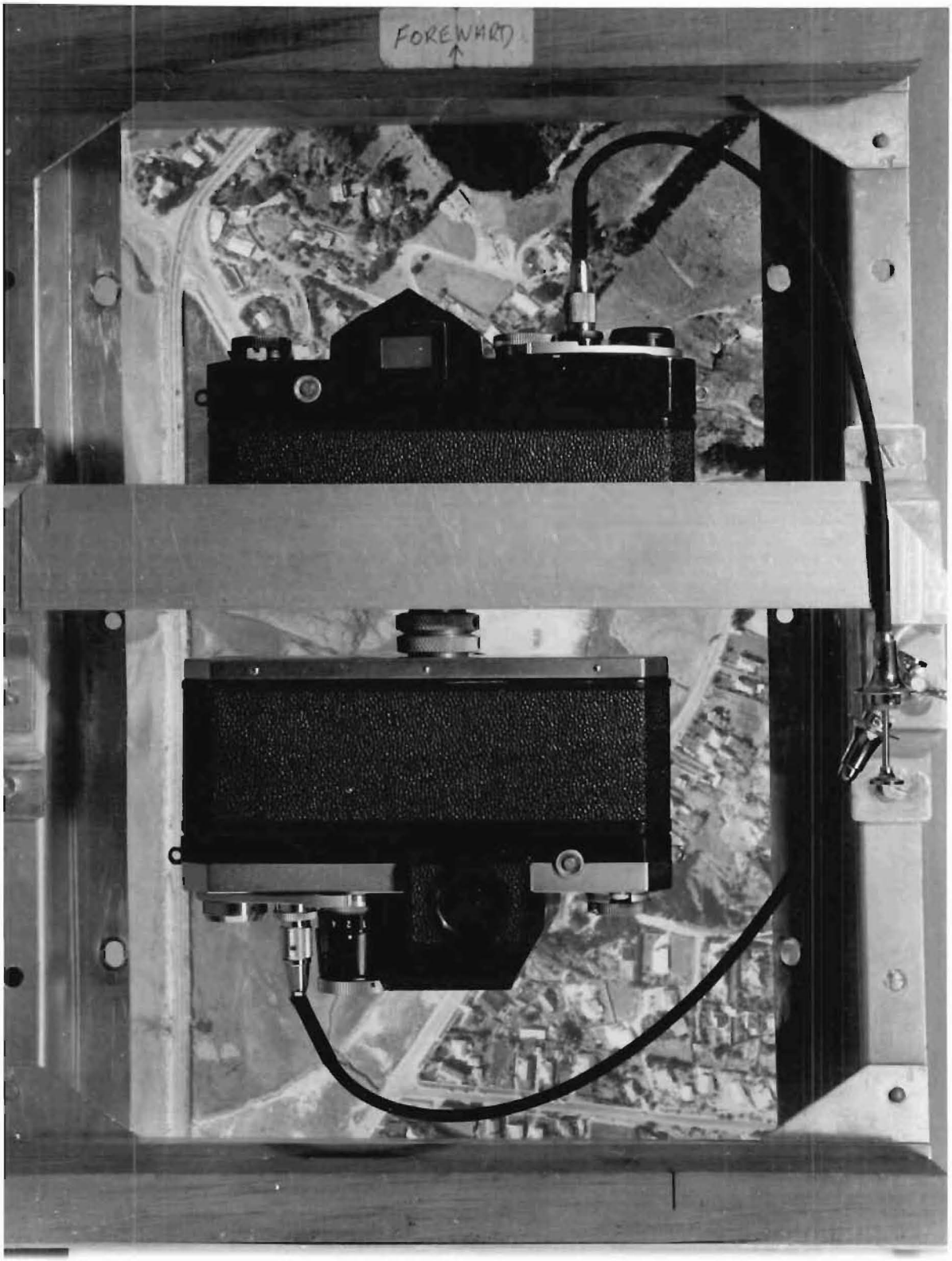

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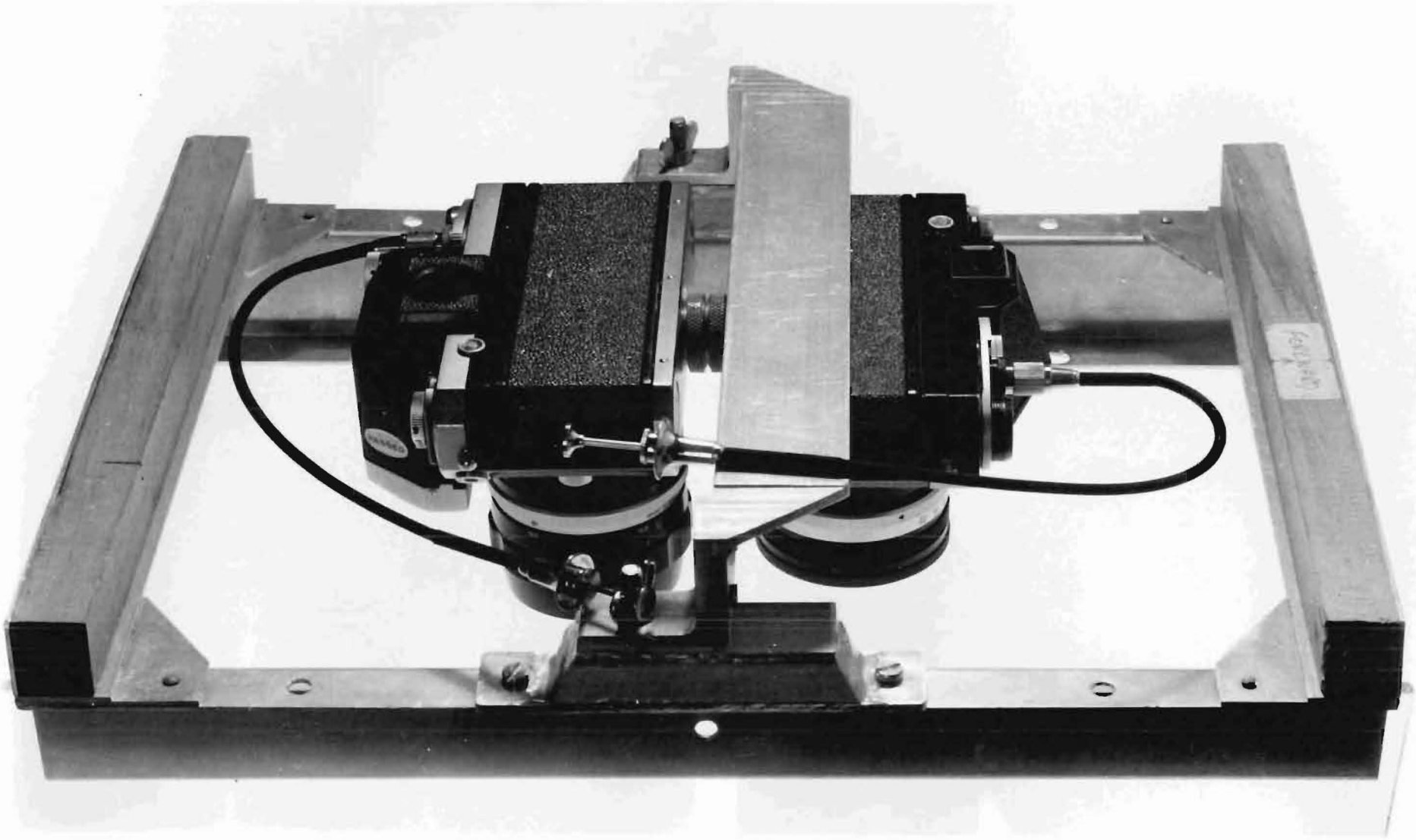
Plate 4.2

Paired 35mm cameras showing the mounts.



(b) Equipment

The photographs were taken from a single engined, high winged Cessna 172 light aircraft in two ways: oblique by means of hand-held cameras through an open window and vertical through a hatch in the floor of the aircraft with the cameras mounted on a shock-absorbent frame (see Plates 4.1 and 4.2).

Two camera systems were used: a Williamson F24 aero camera fitted with a 5 inch lens (127 mm) and taking 5 inch square negatives; and two 35 mm Nikon cameras mounted back to back on the frame and operated simultaneously. Both 35 mm cameras were fitted with a 28 mm wide angle lens, which gave approximately the same angle of view as the 5 inch lens on the F24.

(c) Selection of film

There has been considerable discussion in the literature on the merits of various films. Black and white film is the least expensive, simplest to use and, according to Haack (1962), Olson (1964) and Scott and Tueller (1973), the results are as accurate for vegetation classification as with the more expensive colour films. Some workers have reported greater success with infrared colour film. This is a three-layered false colour film, one layer responding to radiation in the near infrared (700-950 mu) by forming a cyan dye; the second layer responding to the visible green (600-700 mu) producing a magenta dye; and the third layer responding to the visible blue radiation (500-600 mu) producing a yellow dye on the processed film (Tarkington, 1953; Sorem, 1967).

Healthy green vegetation reflects radiation in both the

visible and near infrared regions of the spectrum, resulting in red to magenta tones on the positive film. Dead vegetation has lower reflections in this range, resulting in blue to grey colours on the film (Cochrane, 1970; Colwell, 1968). Infrared colour has been reported as superior to other films for separating species by Anson (1966), Ciesala et al (1967), Colwell (1961, 1967, 1968) and Meyer and French (1968). It has also been used to detect plants under stress or affected by fire, pests, or disease; (Cochrane, 1968; 1970; Marshall, 1968; Meyer and French, 1967). It is claimed by Phillpots and Wallen (1969) and Marshall (1968) that the loss of infrared reflectance precedes colour changes in the visible spectrum. This claim is disputed by Benson and Sims (1967), who suggest that signs of stress are visible on normal colour film and that these are more easily interpreted. Knipling (1969) supports this opinion with the following comment: "Physiological disturbances in plants generally are accompanied by increases in visible reflectance but the direction of change in infrared reflectance is quite variable". Although changes in infrared reflectance may not give the advance warning of stress previously claimed, Cochrane (1969) supports the use of infrared film on the basis of its greater contrast which emphasises differences in reflection, and its greater haze penetration due to the reduced Rayleigh scattering in the longer wave-lengths.

One factor limiting the use of infrared in aquatic studies is the high absorption of infrared radition by water. Fritz (1967) found that one foot of water decreased the red and green layers of the film by half a stop, but decreased the near infrared layer by 1½ stops. Marshall (1967) attributed

some of the colour differences in photographs of aquatic plants to variations in the amount of superficial water. Pestrong (1969), however, noted that: "The presence of vigorously growing organic matter such as algae should image red if present in sufficient quantities at or near the surface".

The films used in this study were: panchromatic "plus X" aerographic film in the Williamson F24 camera; Ektachrome 'X' colour positive and aero infrared colour positive for the paired 35 mm cameras. The latter film was used with a medium yellow filter equivalent to a Nikon Y52.

(d) Survey methods

The vertical photographs were taken on parallel runs across the Estuary orientated parallel to the wind direction to reduce lateral drift of the aircraft. The Estuary could be covered by 3 runs at 8000 feet or 6 runs at 4000 feet, with up to 6 exposures per run. The spacing between runs was determined by the area covered by each photograph at each altitude, allowing for 50% overlap between runs. The flight plan was drawn on an existing map, and a distinct landmark chosen to guide the plane onto the correct path. Several flight plans were prepared to allow for the prevailing winds. On one occasion the exposure interval was determined from the estimated ground speed and the interval set by an automatic timer connected to a motorised back on the camera. This equipment was cumbersome to use in the confined space of the cockpit, and in the short runs over the Estuary proved to be an unnecessary complication. The method usually employed was to make a trial run and time the passage of a landmark across

Plate 4.3

Oblique photograph of the south-west corner of the Estuary in normal colour. November 1971.

Plate 4.4

Same view as in plate 4.3 but in infrared colour. Active algae appear pink.

half the field of the camera viewer. This gave the interval between the exposures which could be checked in the same way during the photographic runs.

Photomosaics were made using the uncontrolled method (Sissam, 1947) where the prints are trimmed to remove the most distorted areas at the margins and matched by means of overlapping ground features. The mosaic was rephotographed and its accuracy checked by projecting it on to a survey map of the area and comparing permanent features such as the roads around the Estuary. Slides of individual photographs could also be projected in the same fashion, and features such as algal beds traced onto the original map. This was more accurate than the photomosaics, as it was possible to compensate for any tilting of the aircraft by tilting the base board of the enlarger or projector. The original transparencies also gave better resolution than the prints.

The Estuary was surveyed by vertical photography in November 1971; March, June and October 1972; February and December 1973. Low level oblique photographs were also taken at these times.

2) RESULTS

The black and white film, having the larger negative, gave greater resolution of features with distinct outlines, but lacked the tonal qualities required to clearly distinguish vegetation. Only the distinct circular shape of the Zostera beds could be clearly delineated.

The normal colour film clearly outlined the large beds of drift green algae but did not detect concentrations below

70-80% cover. Some of the areas of drift near the high tide mark off Humphrey's Drive had a slightly brownish tone (see Plate 4.3). The ground samples showed that these areas contained predominantly bullate (i.e. distorted and blistered) thalli.

On the infrared film the main beds of drift green algae appeared dark red, while the scattered attached plants appeared pink (Plates 4.4 and 4.6). Concentrations as low as 40% cover could be detected. The areas of bullate drift appeared grey to black and where clearly distinguishable from the bright red of the normal drift. Areas where water remained over the algae, such as McCormack's Bay, gave a magenta to purple colour on the infrared film (Plate 4.8).

Discussion of results of aerial photography

The survey technique in this study is very simple and relatively crude compared with the sophisticated techniques used in large scale aerial mapping and it would be less suitable for areas where no survey maps were available to test the accuracy of the photomosaics. In this study there was a good fit between the photomosaics and the existing map for the stable features such as roading patterns (see Plate 1.3).

This method was successful in mapping recent changes in the shore-line and drainage pattern and gave a very good overall view of the vegetation. To collect comparable data from ground surveys would have required considerably more time and man-power.

Of the films used, infrared colour was the most successful as it was able to detect lower concentrations of algae and gave a greater contrast between the moribund,

bullate drift and the active plants. This is similar to the results given by Cochrane (1969). The sensitivity of the infrared film to surface water also made it more suitable for surveys of the drainage pattern. The absorption of infrared wave-lengths by water provided the main problem in interpreting colour tones on the infrared film. The shift towards purple colours in algae covered by water noted by Marshall (1968) was also found in this study. Normal colour film, although generally less useful, was needed to assist in interpreting the colour tones in the infrared film, especially in areas covered by water. As the colours on the processed film could vary with different film batches and processing, colour comparisons should only be made between photographs taken at the same time with the same film batch and processed at the same time. Comparisons of photographs taken at different times of the year may also be affected by colour shifts as a result of different light attitudes (i.e. angles). The photographs in this study were taken between 11 a.m. and 2 p.m., which would reduce this problem.

The aerial photography provided a very useful addition to the ground truth data of the field study. The time saved and the additional information gained more than compensated for the cost of plane hire and of films.

Plate 4.5

Vertical photograph of areas C, B and F near the mouth of the Heathcote River. Normal colour from 4000 ft in June 1972.

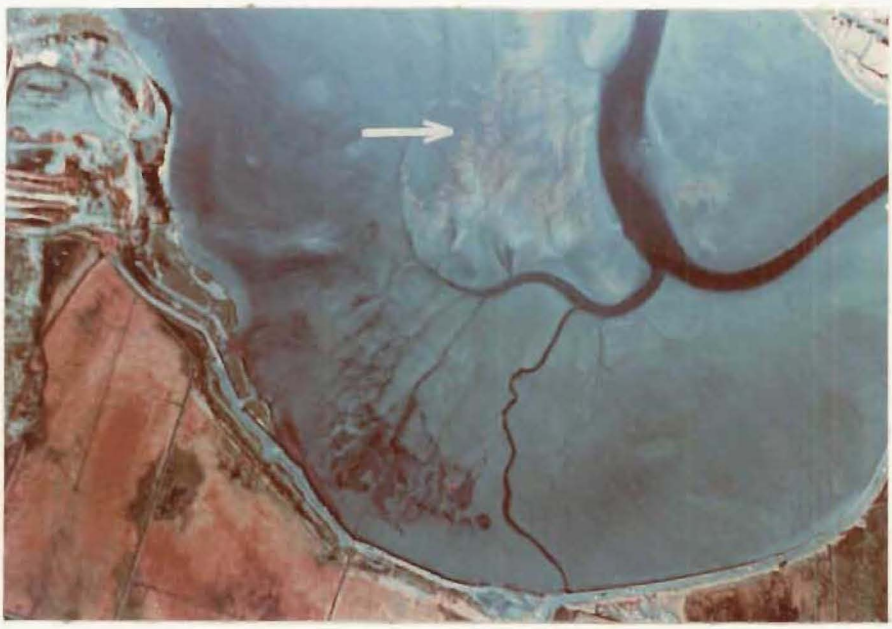


Plate 4.6

Same area as plate 4.6 but in infrared colour. Note area of pink in centre indicating active algae (marked by arrow).

Plate 4.7

Vertical photograph of McCormack's Bay, normal colour, 4000 ft.

Plate 4.8

Same area as plate 4.7 but in infrared colour. Note the lack of infrared reflectance from the submerged algae (marked by arrow).

CHAPTER V

DESCRIPTION AND TAXONOMY OF THE ALGAL SPECIES

The four common species, Ulva lactuca, Enteromorpha ramulosa Hooker, Gracilaria secundata and Chaetomorpha linum are described in order of ecological importance. Due to its uncertain taxonomic status, Ulva has been examined in greater detail than the more clear cut and less important species. The less common algae are listed at the end of this chapter.

1) ULVA SPECIES

(a) Introduction

As mentioned above, the specimens on the Estuary do not consistently key out into the taxa recognised by Chapman (1956). Many keyed out to Ulva lactuca L, or its varieties rigida and lacinulata as recognised by Chapman, but a number resembled U. latissima L., and Ulva laingii Chapman. A search of the literature indicated a wide range of opinion on the significance of these forms and their taxonomic status. Many workers regard U. lactuca L. as a composite cosmopolitan species with a number of different forms; others recognise several distinct varieties within U. lactuca, while some recognise them as separate species. With the exception of Bliding (1968) on the European taxa, no-one has attempted the extensive interbreeding experiments necessary to establish the

exact status of these taxonomic units.

There are five separate forms in the lactuca complex which have been widely recognised at various levels:

(i) The short frond, wider than long, ovate and variously dissected. This has been described as U. lactuca L. by Newton (1931), Lucas (1936), Smith (1944) and Chapman (1956).

(ii) A second form similar to the one described above, but with lanceolate fronds with dentate margins has been recognised as U. lactuca var lacinulata (Kützing) Taylor (1960) and var lacinulata (Kützing) Hauck by Feldman (1937) and Chapman (1956). Taylor does not cit Hauck but appears to be using the same combination.

(iii) An exposed coast form with a thicker, stiffer thallus and distinct stipe has also been widely recorded and described as U. lactuca var rigida Hauck by Collins (1903), Laing (1926), Hamel (1931), Newton (1931), Taylor (1960, 1962) and Chapman (1956). It was described as U. rigida C. Agardh by Hooker (1867), Cotton (1909), Setchell and Gardner (1920), Feldman (1937), Dangeard (1958), Papenfuss (1960) and Bliding (1968).

(iv) A large expanded thallus, which becomes free floating early in its growth. This has been classified as:

U. lactuca var latissima (L.) De Candolle, Collins (1903), Le Jolis (1863), Setchell and Gardner (1920), Laing (1926). U. latissma L., Hooker (1867), Cotton (1909) and Chapman (1956).

Ulva expansa (Setchell), Setchell and Gardner (1920), Smith (1944).

U. gigantea (Kützing) Bliding (1968).

Note: Papenfuss (1960) has suggested that the type U. latissima L. is in fact representative of Laminaria saccharina (L) Lamoroux.

(v) A fifth form consisting of a very contorted bullate thallus, usually fragmented and drifting. This has been recognised as Ulva mesenteriformis J. Agardh (1882), Detoni (1889); and U. lactuca var mesenteriformis (Roth) by Collins (1903) and Taylor (1960, 1962). A specimen in the herbarium of the Botany Division, Department of Scientific and Industrial Research, Lincoln, New Zealand, collected by L.B. Moore from the Collingwood Estuary in 1947 was determined as U. mesenteriformis by Chapman in 1949. However, Chapman (1956) proposed U. laingii to include the New Zealand specimens of this form. The type was collected by R. Laing from the Heathcote Estuary. Chapman suggested that the U. bullosa (Roth) recorded by Hooker (1867), and U. cornucopiae recorded in New Zealand by Laing (1926) may belong to U. laingii.

The lack of agreement on the status of the forms in this group of Ulva can be related to the polymorphic nature of this genus and the lack of stable morphological characters on which to base a convenient key. This variability has made many workers cautious about their identifications; Womersley (1956) for example prefers to treat all of the forms on the South Australian coast as U. lactuca L. until interbreeding tests are carried out. The following comment by Setchell and Gardner (1920) points to some of the difficulties: "The habit, size, colour and even the character of cell depends so much on the age and environment of the specimen, that it is possible to trace a series from the quiet water inside a point of land

to the exposed localities outside of it which may include all the forms and intermediate conditions between the most distinct species as yet proposed under this genus".

The most definitive work on this genus is Bliding's (1968) for the European taxa. The species were delineated on the basis of anatomy and life history and checked by cross-breeding experiments. This resulted in a number of new species and new combinations, which cut across many of the early classifications based solely on morphological features.

Ulva lactuca L, as recognised by Bliding, includes the Enteromorpha linza-like fronds and the deeply laciniated palmate thallus resembling Linneaus' original specimen as described by Papenfuss (1960). The margin is never dentate. Anatomical features include cells arranged in short curved rows, cell shape varying from rectangular, quadrangular to rounded, rarely slightly polygonal, averaging 18 x 13.5 μ in surface view. The membrane varies from 48 μ thick at the margin to 60 μ in the centre and up to 100 μ in the rhizoidal zone near the stipe. Cell heights follow a similar pattern, that is, 16, 26, 27-31 μ respectively. The distribution is given as mainly Northern European.

Ulva rigida (C. Agardh) Bliding is characterised by "tooth-like generally microscopic protruberances on the margin of the disc", "ball-shaped" marginal cells in the upper part of the plant. In other respects the anatomy and morphology is similar to U. lactuca L. It is described as the most common species on the Mediterranean and European coasts. Bliding regards U. lactuca var rigida (Agardh), (Le Jolis, 1863; Hamel, 1931; Newton, 1931) and Ulva lactuca L. form lacinulata (Kützing), (Hauck, 1885; Feldman, 1937) as agreeing with

U. rigida (C. Agardh).

Ulva gigantea (Kützing) Bliding, is characterised by a thallus 2-4 decimetres long and 1.5-3 decimetres broad, remarkably thin in the upper part and lacking the microscopic tooth-like protruberances of U. rigida. In section the membrane of the upper thallus is 28 μ, the central region 35 μ and the rhizoidal zone 80 μ. Cell height varies from 12 μ at the margin to 22-30 μ near the base. Bliding includes Phycoseris gigantea Kützing, Ulva lactuca L. (sensu Thuret, 1894) and U. latissima L.?

U. gigantea.

Bliding (1968) makes no mention of Ulva mesenteriformis or bullate forms. If Bliding's work is applicable to the New Zealand specimens, Chapman's Ulva lactuca L. var rigida and Ulva lactuca var lacinulata could be included in U. rigida. Chapman's U. latissima L. could fit into U. gigantea (Kützing) Bliding, or U. lactuca (L.) Bliding. This would be based on the assumption that this section of the genus is cosmopolitan. As the cross-breeding experiments necessary to establish this point are outside the scope of this study, comparisons between New Zealand and European specimens should be treated with caution bearing in mind the geographical isolation of New Zealand.

(b) Description of Ulva species from the Estuary

(i) Morphological types: In spring the most common Ulva plants are small thalli 2-10 centimetres in length consisting of 2 to 6 ovate fronds radiating from the base (Plate 5.2). The stipe is short and inconspicuous. The plants are most commonly attached to Chione or Amphidesma

shells, both dead and living. During spring and early summer the fronds elongate, becoming more laciniate (Plate 5.3). Often one frond will grow much longer than the others. Plants attached to secure substrates such as large rocks may exceed 1 metre in length (Plate 5.4), while those attached to shells (Plate 5.3) have a maximum length of 60 centimetres.

In summer and early autumn the large plants tend to fragment and often the buried attached shell will be uprooted and the whole plant will drift within the Estuary. In late summer very large expanded sheet-like thalli become common in the Estuary. They float freely at high tide and form large drifts in the mid-tidal zone at low tide. Considerable quantities also accumulate at the high tide zone where they decompose, producing a foul smell. These large sheet-like thalli are present all year round in McCormack's Bay. On the Estuary the patches of drift decrease in area during winter, and in 1972 disappeared completely. The size of the thalli within the drift also decreases during winter. Large attached thalli are absent during winter; only the small plants (2-5 centimetres) remain, often attached to the inside of empty shells and in sheltered areas on the retaining walls around the shore line (Plate 5.1).

In the area off Humphrey's Drive north of the Heathcote River a bullate, contorted thallus is common in the drift. It varies from moderately folded or bullate, not very different from the normal thalli, to small, dark green, extremely crumpled and folded. The latter specimens become more common in autumn (Plates 5.5 and 5.6).

The thallus margin of the small plants has microscopic tooth-like projections which are particularly noticeable in

the lower part of the frond. In the expanded region of the larger fronds, and in the drift from these, the dentations are less obvious or lost completely.

In the permanent open quadrats it was possible to follow individual plants through from the small palmate form to the large elongated form, but it was not possible to follow individual plants after they detached. An attempt was made to completely enclose quadrats with fine netting over a steel frame but unfortunately, the frames placed in the more accessible areas disappeared although they were firmly secured. Their suitability as fishing nets apparently did not go unnoticed by some members of the public. A number of the remaining frames were distorted by large quantities of algae piling on the top of the netting, so that the drift algae inside the nets may have come from outside. In the two frames which remained more or less intact, the attached plants were followed into the drift state. The fragments continued to grow and sheets of up to 30 metres had developed before the netting collapsed. (See Field results for Plates 6.16, 6.17 and 6.18).

(ii) Anatomy of specimens from the Estuary: Specimens of all the morphological types were examined and the cells described in surface view and in cross-section. Sections were cut from the expanded, central and stipe regions of the thallus. The results are summarised in Table 5.1.

In surface view the cells of the smaller plants are rectangular (7-9) μ x (11-14) μ in the expanded region of the thallus (Plate 5.7). The cells increase in size towards the stipe of the smaller plants and increase generally with increasing thallus size. In the large attached fronds and the

Table 5.1 Cell sizes of Ulva lactuca in μ

Morpho-logical type	Position of section	Membrane thickness		Cell height		Cell width		Cells in surface view
		Range	Mean	Range	Mean	Range	Mean	Range
Small attached thalli Plate 5.2 (A-C)	Margin	38-42	40	10-12	10.5	5-7	5.8	7 x 11
	Centre	49-52	50	10-12	11	7-9	8.2	7 x 11
	Stipe	65-70	67	11-13	12	7-9	8.1	7 x 11
Larger attached thalli Plate 5.2 (H)	Margin	50-54	52	17-18	17.4	9-14	12	(9-10) x (12-14)
	Centre	70-75	72	24-25	24.5	10-12	11.1	(8-12) x (12-16)
	Stipe	105-110	106	27-30	28	10-15	12	(14-16) x (18-22)
Expanded attached form Plate 5.2 (F)	Margin	71-74	73	22-25	23	14-16	15	(11-14) x (15-20)
	Centre	74-77	76	22-25	23	14-16	15	(11-14) x (15-20)
	Stipe	175-180	176	17-20	19	6-12	*	-
Large ribbon-like thallus Plate 5.3	Margin	52-54	53	17-17.5	17	10-14	12	(10-11) x (13-14)
	Centre	70-73	72	20-22	21	12-14	13	(10-11) x (13-14)
	Stipe	130-135	133	16-18	17	11-14	13	(10-11) x (13-14)
Bullate form Plate 5.5 (E)	Margin	70-72	71	15-18	16	10-12	11.1	(11-14) x (15-20)
	Centre	73-77	74	17-22	18	11-12	11.3	12-17 (dia)
Bullate from area C Plate 5.5 (A)	Margin	52-54	53	13-14	13.5	7-9	8	(11-14) x (15-20)
	Centre	52-55	53	16-18	17	9-13	11.1	5.8-8.4 (dia)
Large expanded drift plant	Margin	70-73	71	18-21	19	11-13	12	10-16 (square)
	Centre	74-77	75	24-25	24.3	12-14	13	(7-9) x (10-13)

* Cells pear shaped in T-S. 10-12 μ at base and 6-8 μ at the top.

All means determined from five specimens of each type.

drift thalli, the cells become more quadratic or rounded. In the bullate form the outer cell wall is similar in shape and size to that of the normal plants, but the lumen becomes rounded and much smaller, (5.8-8.4 μ mean = 6 μ) (see Plate 5.10). The dark green patches on the fronds are associated with epiphytic diatoms on the surface. These regions tend to be more tightly folded or contorted than areas lacking diatoms.

In section the membrane increases in thickness towards the stipe and increases generally with the size of the thallus. The cells follow the same trend in size, but vary in shape (see Fig. 5.1). Cells of the smaller plants are almost square in section in the expanded region of the thallus, but tend to be more rectangular in the stipe region. In the larger plants the cells near the margin are square to rounded in section; in the central region they are rectangular, becoming more elongated towards the base. In the rhizoidal region of the stipe the cells tend to be elongated, but are variable in shape (Plate 5.8). They often taper towards the top or bottom, producing a 'pear' shaped cell. The cells in the bullate thalli vary in shape according to the degree of contortion of the thallus (Plate 5.10). In the moderately bullate thalli they are similar in shape and size to those of the normal thalli. In the very contorted regions the cells are extremely variable, being rounded, pear-shaped or elongated. In some areas the two layers split to form a blister. The cells in these regions are very similar to those in the rhizoidal zone of the normal thallus (Plates 5.8 and 5.10). All the above cell types can be found in the same plant.

(iii) Identification of specimens: From the descriptions given in Chapman (1956), the attached forms of

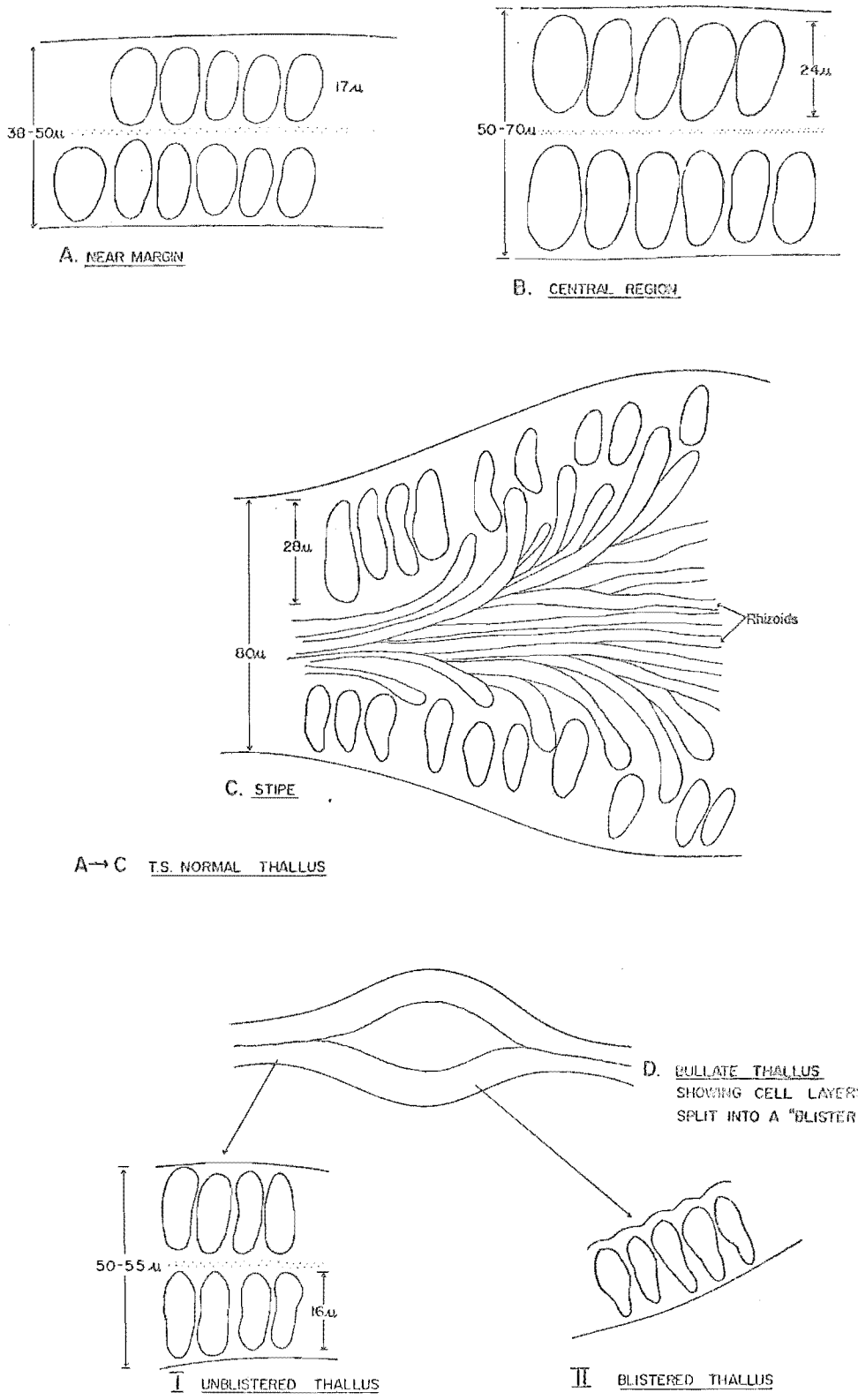


Fig. 5·1. Representative cell shapes and sizes of Ulva lactuca.

the Estuary most closely resemble U. lactuca var lacinulata. From Bliding's descriptions, these plants resemble U. rigida on the basis of the dentate margin, the ball-like cells at the margin and the tapering cells in the rhizoidal zone. These tapering cells, however, are less than half the size quoted by Bliding. Also, the tooth-like margin is only well developed in the small plants or at the base of the longer plants. From a variety of specimens collected on Banks Peninsula, Kaikoura and Timaru, tooth-like projections on the margin are present on very dissimilar specimens and may be present or absent on very similar ones. This feature appears to have less taxonomic significance in the New Zealand specimens than in the European plants as described by Bliding. The variability of the margin and the small size of the cells in the rhizoidal region does not support the classification of U. rigida. It is noteworthy that the description of Linneaus' original specimen as quoted by Papenfuss (1960) is similar to the material found on the Estuary, including the presence of dentations on the lower margins of the fronds, but not on the upper part of the frond.

If the expanded sheets develop from detached plants or fragments of attached plants as results of the permanent quadrats indicate, they cannot be attributed to a different taxon. It is, however, not proven that all the drift thalli come from the attached plants as described above. This applies particularly to the bullate thalli which often differ in morphology and anatomy from the rest of the specimens. The occurrence of thalli intermediate between the normal drift and the very bullate thalli, and of intermediate regions within the thallus of individual plants, suggests the

possibility of the bullate thallus developing from the normal form. Chapman (1956) noted that Ulva laingii occurred in areas of stagnant water or in association with fresh-water influxes and suggested that it might be an ecological form rather than a separate species. The localities of the bullate form on the Estuary are areas of low salinity usually around 15°/oo whereas the salinity of most other areas is over 20°/oo. To test the effect of salinity on the morphology, specimens of attached and drifting plants with flat thalli were grown in the laboratory for a period of three months in water of 15°/oo salinity taken from the locality of the bullate plants, of 20°/oo taken from the centre of the Estuary and of 10°/oo taken from upstream of the Ferry Mead Bridge at high tide.

The specimens in the 10°/oo water did not survive, those in 20°/oo grew normally, but the specimens in 15°/oo after one month began to develop the bullate thallus and split cell layers typical of the naturally occurring bullate thallus. This happened in both the drift and attached plants. At the end of three months the specimens were noticeably bullate in appearance and were similar in anatomy to the naturally occurring bullate form (Plate 5.5).

This strongly supports the hypothesis that the bullate thallus develops from the normal thallus in response to the water overlying the mud fluts near the mouth of the Heathcote River. It also suggests that low salinity may be the cause, but a number of other factors may also change in this area. The extremely contorted thalli invariably have a dense covering of epiphytic diatoms, which may contribute to the anatomical changes. It is also possible that the diatoms only occur on thalli which have been in the drift for a long period

and that it is the length of time spent in this region rather than the diatoms which intensifies the contortions of the thallus. The current pattern on the Estuary tends to wash drift algae from other areas of the Estuary into this region. Here the same debris could be found from month to month, suggesting that the individual thalli may remain there for long periods. The low reflectance of infrared radiation of the bullate plants supports the suggestion that they are moribund.

Collections have also been made in other estuarine environments in the South Island - at the Moutere Inlet, Nelson Haven, Collingwood Estuary, the Motueka and Pohara beaches in the Nelson district, and Blaketown Lagoon, Greymouth. Plants similar to those found on the Avon-Heathcote Estuary have been found at all these locations. As the observations in this study have been made from a small part of the New Zealand representatives of this genus, and did not include cross-breeding experiments, it is not possible to make any general taxonomic conclusions. The evidence does justify, however, the placing of all the specimens on the Avon-Heathcote Estuary in the same species. Ulva laingii Chapman therefore appears to be an ecad rather than a separate species. This possibility was suggested by Chapman when he proposed the species.

Although it is reasonably clear that there is only one species on the Estuary, it is not clear which species name is most appropriate. The name proposed here is Ulva lactuca L., based on the similarities with the type as described by Papenfuss (1960). With the exception of the marginal dentation, it also agrees with U. lactuca L., sensu Bliding

(1968). There are, however, some similarities between the Avon-Heathcote specimens and U. lactuca var lacinulata (Kütz) Hauck, as described by Chapman (1956), and U. riqida (Agardh) Bliding (1968).

2) ENTEROMORPHA RAMULOSA (Smith) Hooker

This species is the most common Enteromorpha on estuaries in the South Island of New Zealand and can be separated from related species such as E. clathra by its distinctive and prolific branching. Chapman (1956) describes two varieties and three forms of E. ramulosa based on the degree and type of branching. Bliding (1963) places all the European forms into E. ramulosa (Smith) Hooker.

There are four morphological forms on the Estuary:

(i) A small low growing plant which forms a mat over the rocks on the retaining walls around the shore. This form is sparsely branched but plants grown in the laboratory have increased in size and have become more prolifically branched as described for form (B) (Plate 5.11).

(ii) Small plants up to 15 centimetres long, with a number of main branches radiating from the base (Plate 5.12a). The main branches have a large number of thin tapering branchlets and small spines, and are usually attached to shells. This form agrees with Chapman's description of f. robusta.

(iii) Large plants from 15 to 30 centimetres have flattened main branches often dilating towards the tip (Plates 5.12b and 5.13). There are fewer lateral branchlets and spines than in the smaller plants. This type fits Chapman's description of var acanthophora, although the attenuation of

the branches is not as consistent as recorded by Chapman. These plants are also normally attached to shells.

(iv) The fourth type is the free floating form. This is similar to the large attached form, but generally has wider branches (Plate 5.12c).

All four forms could be included in E. ramulosa as described by Bliding (1963). The reduction in the number of spines in the older summer plants was also noted by Bliding.

The cell sizes are generally larger than those described by Chapman (1956), but within the range recorded by Bliding. The cells are unordered, quadratic to rounded in surface view and measure 13-17 µ across with two to three pyrenoids per cell. In transverse section the membrane is 46-52 µ with the inner and outer walls of equal thickness. The cells are 29-30 µ high and 8-9 µ wide. The dimensions and shapes are similar for all morphological types.

Swarmer release occurred from the attached plants in summer and early autumn.

All of the specimens on the Estuary can be placed in E. ramulosa with reasonable confidence, but it is not clear which of Chapman's subdivisions would be most appropriate.

A second species, Enteromorpha intestinalis, has also been recorded by Webb (1965) and Cameron (1968) from near the mouth of the Avon River. Cameron described this species as large floating masses in the impounded area of McCormack's Bay in 1968. In 1969 at the same locality the present author found large masses of Chaetomorpha linum, but no E. intestinalis.

3) CHAETOMORPHA LINUM (Mueller) Kůtz
LOLA LITOREA (Cooke) Chapman

Chaetomorpha linum was recorded as large masses of floating filaments (Plate 5.16) in the impounded area of McCormack's Bay by Rosenberg (1965). Cameron may have confused this species with E. intestinalis in 1968.

The material examined in this study fits the general description of C. linum, but also resembles Lola litorea as described by Chapman (1956). The cell shape is generally more similar to that portrayed in Chapman (1956), Fig. 119, but the "barrel" shaped cells described as characteristic of Lola are not consistently present. The cells were generally $1\frac{1}{2}$-2 times as long as broad, but varied from 100-190 μ in width to 150-370 μ in length. These dimensions could fit either species.

As the published descriptions are not adequate to place this material into either species, it is proposed to use the name already established for this locality. As this area was being reclaimed by the Christchurch City Council, the matter could not be pursued.

4) GRACILARIA SECUNDATA Harvey f. pseudoflagellifera May 1948

The taxonomy of this species was discussed at length by Ratnasabapathy (1963) from material in Brookland Lagoon to the north of Christchurch. As a M.Sc. thesis on the Gracilaria population in the Estuary was in progress, this alga has not been covered in detail in the present study.

G. secundata occurs as a sparse population over much of the Estuary attached to the same substrates as Ulva and

Enteromorpha. It occasionally reached very high density during autumn in isolated clumps near Humphrey's Drive. The lower fronds in the clumps were buried in the mud up to 10 centimetres. The upper fronds often showed signs of bleaching, and no reproductive plants were found in the clumps whereas cystocarpic plants were reasonably common over the rest of the Estuary in autumn (Plates 5.17 and 5.18).

Detached Gracilaria was also found among the drifting Ulva and Enteromorpha, but these plants did not appear to continue to grow in the detached state.

5) LESS COMMON SPECIES

(a) Blidingia minima

Found on the retaining wall above the low growing Ulva and Enteromorpha. B. minima is morphologically similar to E. ramulosa but has a basal attachment disc.

(b) Porphyra columbina

Found attached to rocks and shells near Beachville Road and further seawards in the mud to high tide zone.

(c) Gigartina circumcincta

In the same area as P. columbina but near the low tide zone.

(d) Ulothrix sp.

Occurred occasionally near the mouth of the Avon River. Only detached filaments would found suggesting a freshwater origin.

(e) *Polysiphonia sp.*

In crevices at the base of the retaining wall and in McCormack's Bay; possibly two species.

(f) A number of species from outside the Estuary were found in the drift including:

Durvillea antarctica

Carpophyllum maschalocarpum

Macrocystis pyrifera

Plate 5.1

Prostrate Ulva lactuca from the retaining wall, May 1973.

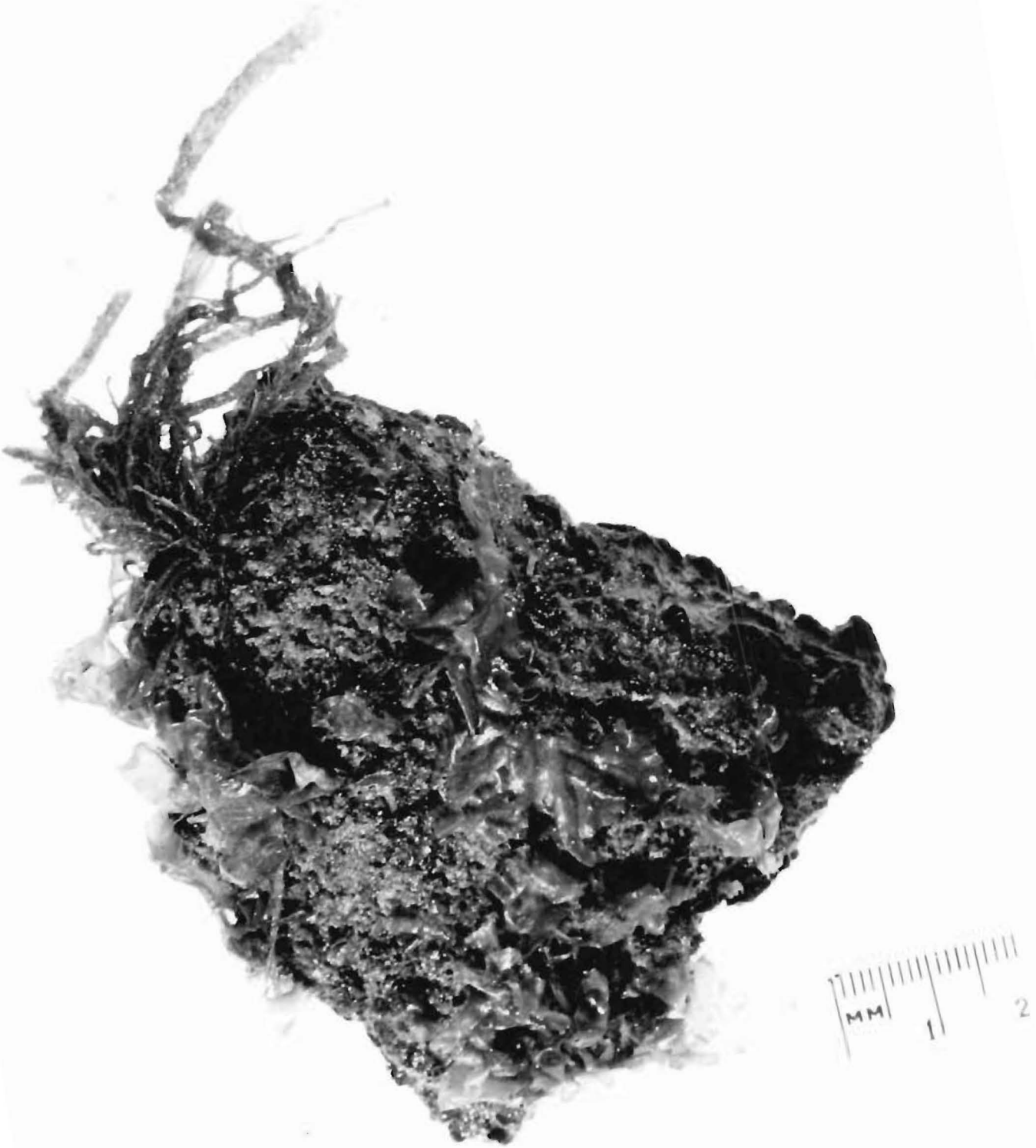

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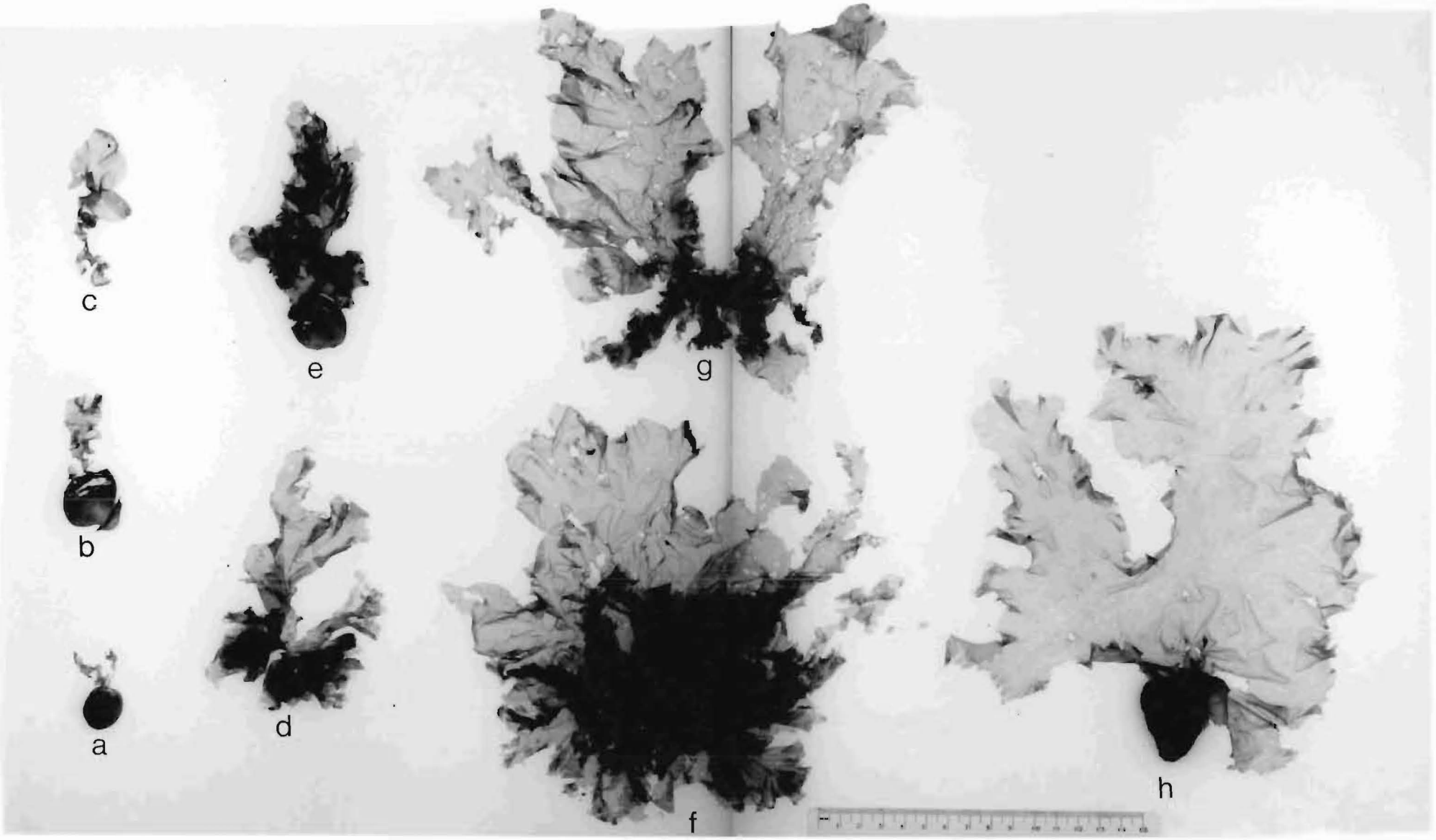
Plate 5.2

A typical range of *Ulva lactuca* found attached to *Chione* shells, November 1973.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b
c
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e
f
g
h

Plate 5.3

Ulva lactuca found attached to a Chione shell showing the ribbon-like thallus with ruffled margin, December 1973.

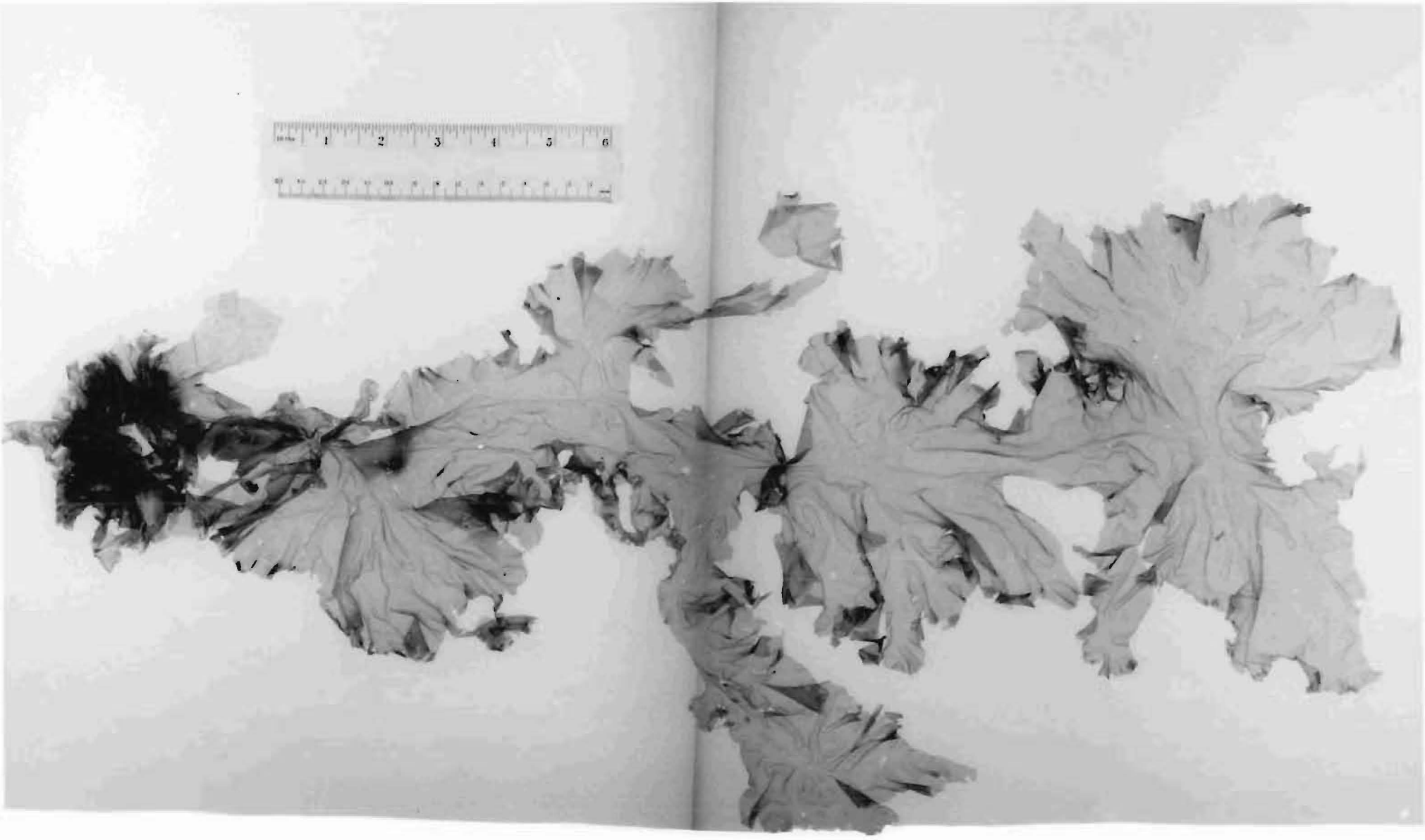

Plate 5.4

Very large *Ulva lactuca* attached to a rock showing perforations from grazing, March 1973.

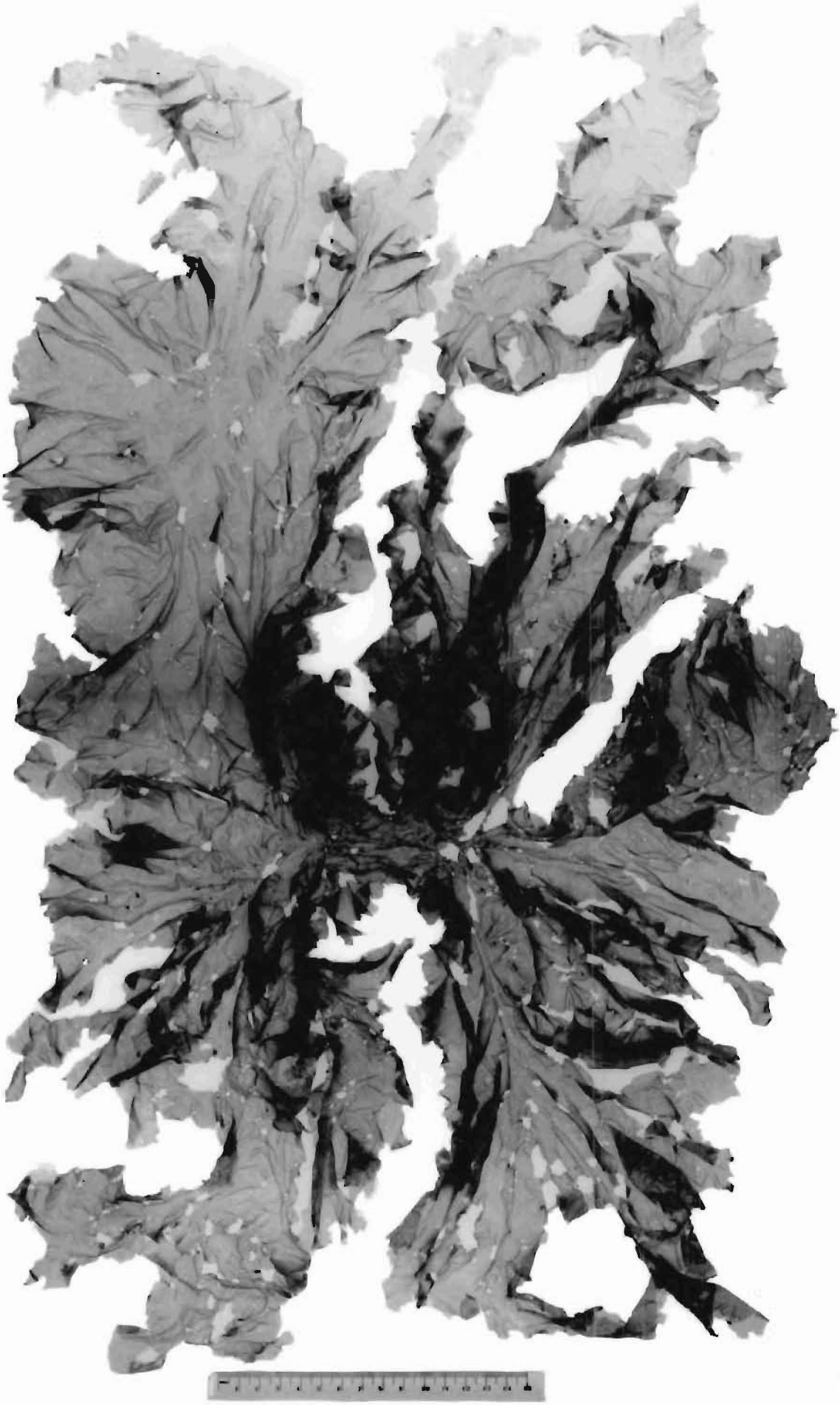

Plate 5.5

Bullate thalli of *Ulva lactuca*.

A and B collected from sample area C.

C, D and E normal plants subject to low salinity in culture.

Bullate nature most clearly shown in E.

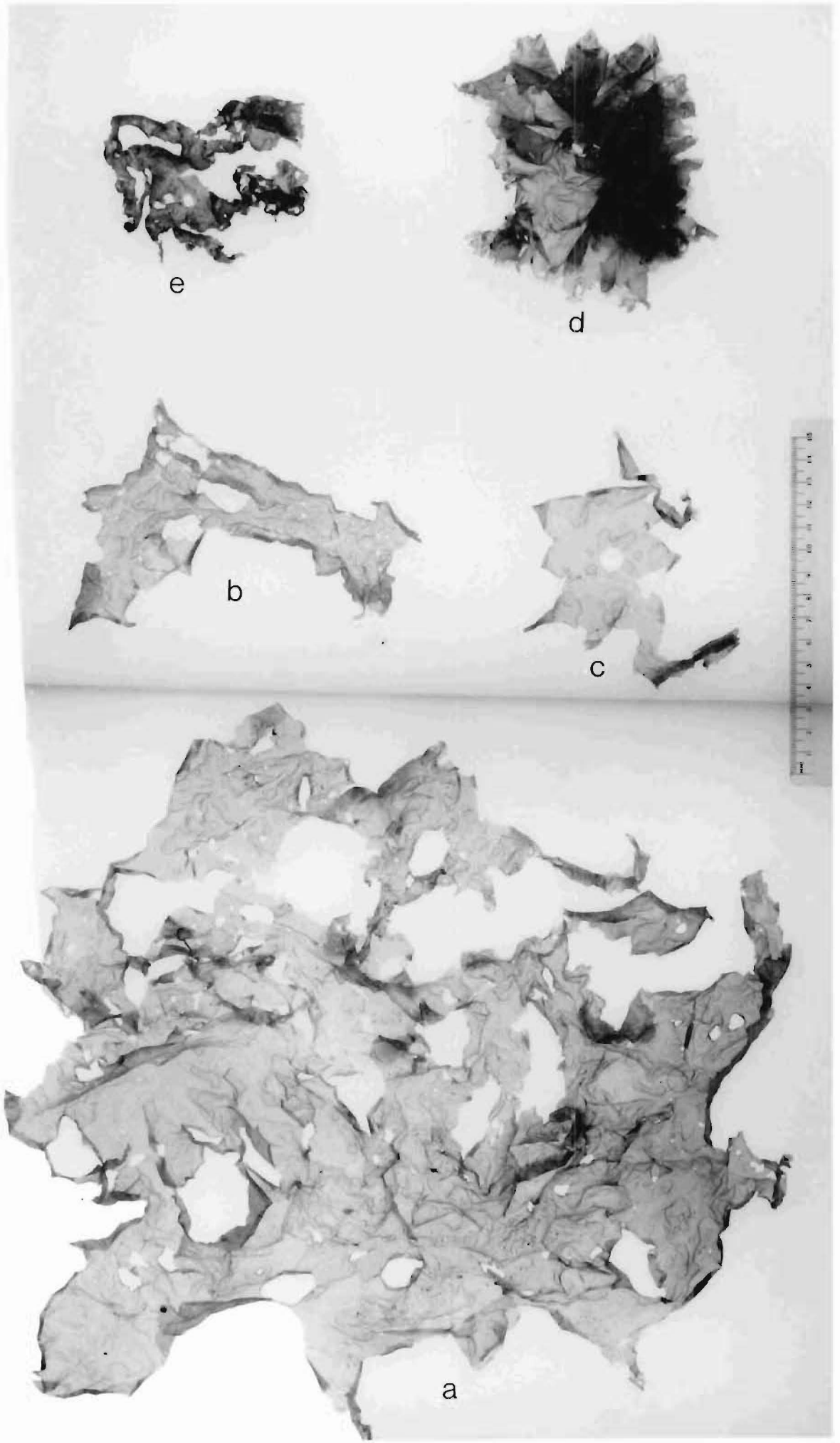
e
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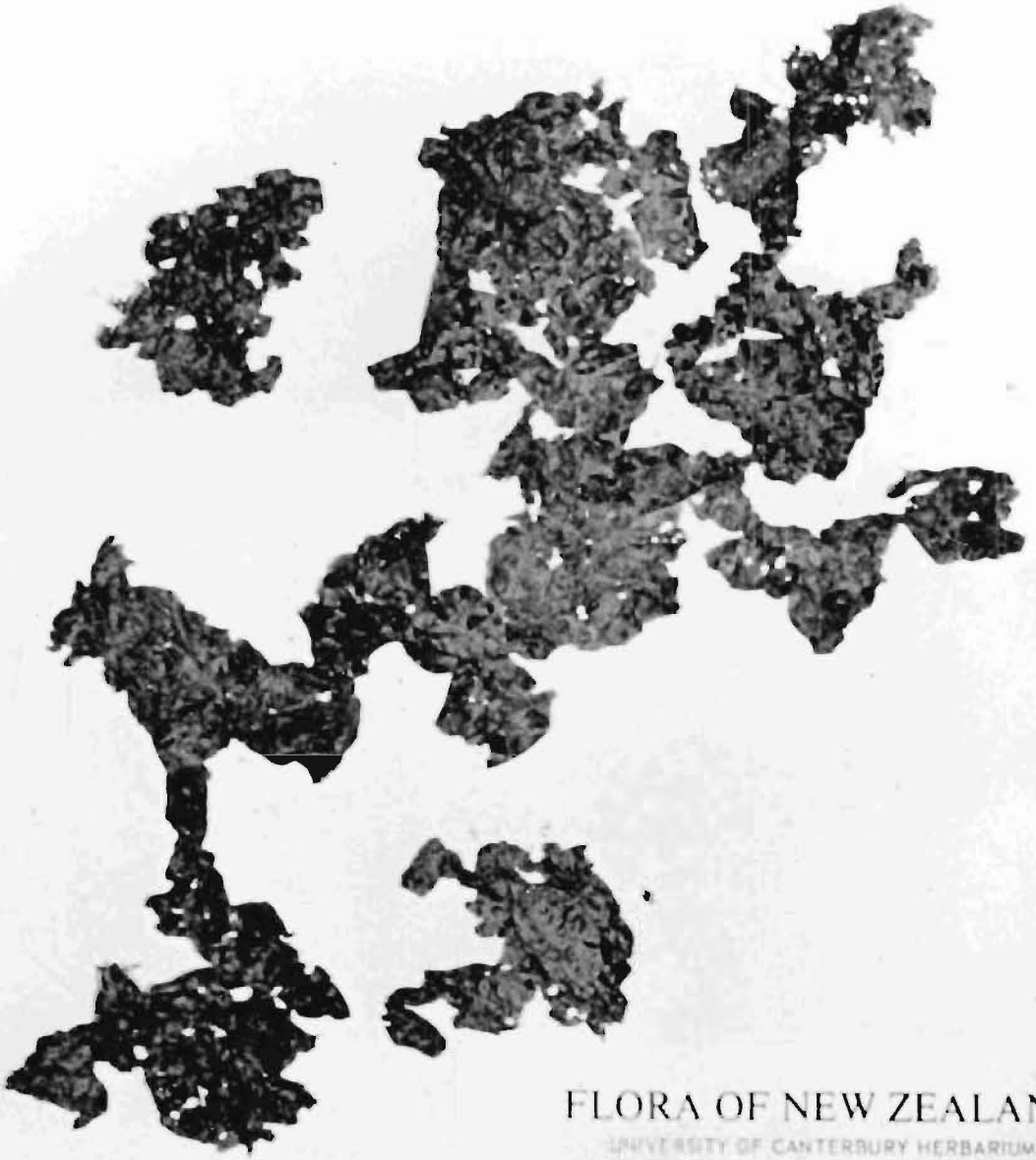
Plate 5.6

Herbarium sheet of bullate Ulva lactuca.



FLORA OF NEW ZEALAND

UNIVERSITY OF CANTERBURY HERBARIUM

Ulva lanosa? (Chermsa).

LOCALITY Avon - Heathcote Estuary.
off Humphrey's Drive.

HABITAT Free floating in large drifts.

ALTITUDE DATE 24.2.77.

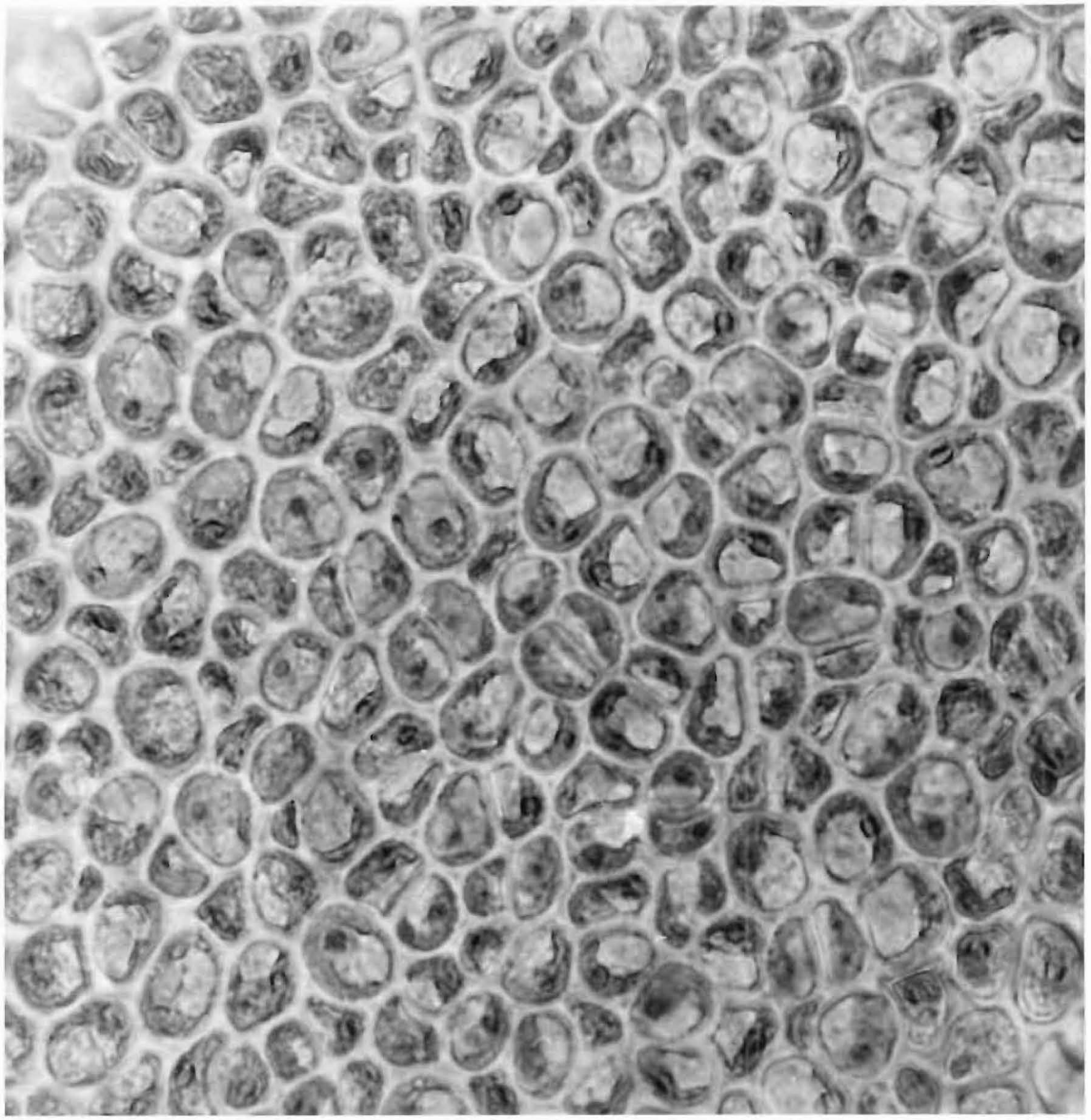
COLLECTOR D. A. Steffensen.

DETERMINED BY D. A. Steffensen. NO

REMARKS

Plate 5.7

Surface view and transverse section from the expanded part of the thallus of specimen (G) Plate 5.2, also showing cell division.



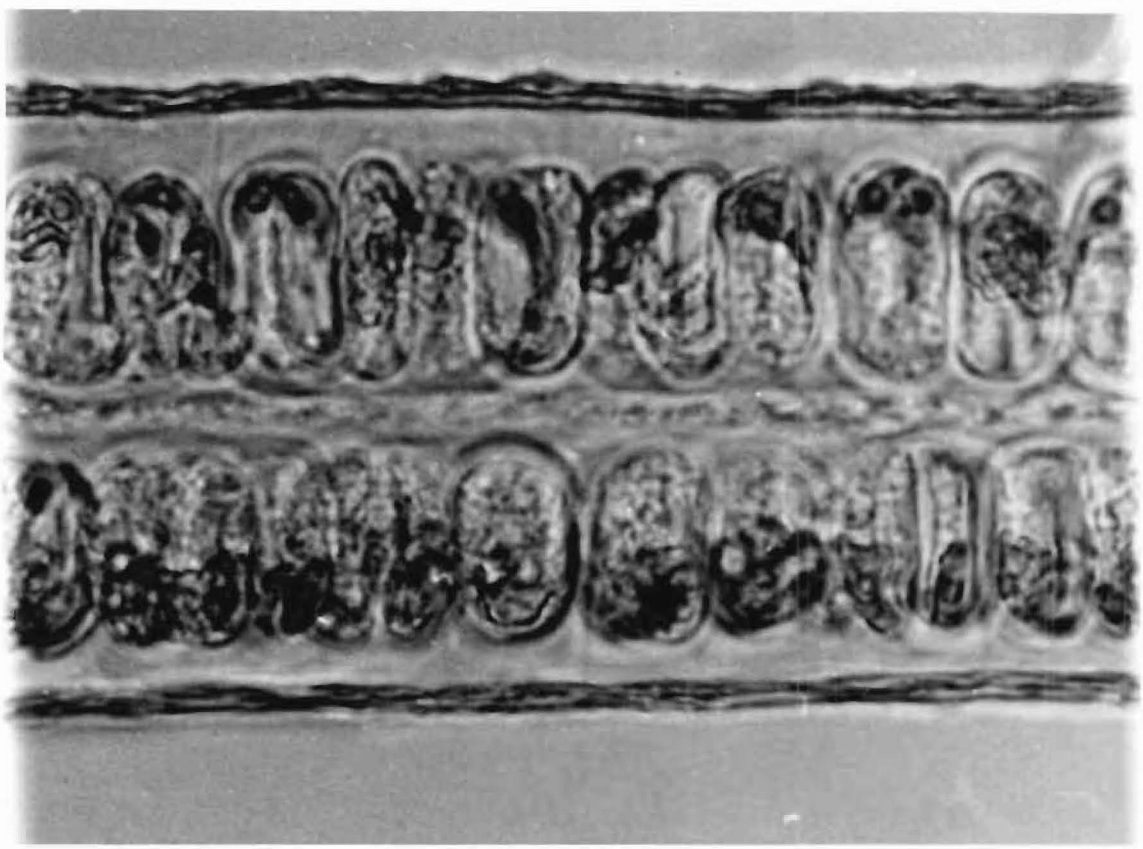


Plate 5.8

Transverse section of stipe of specimen (E) Plate 5.2 showing disruption of the cell layers by rhizoids.

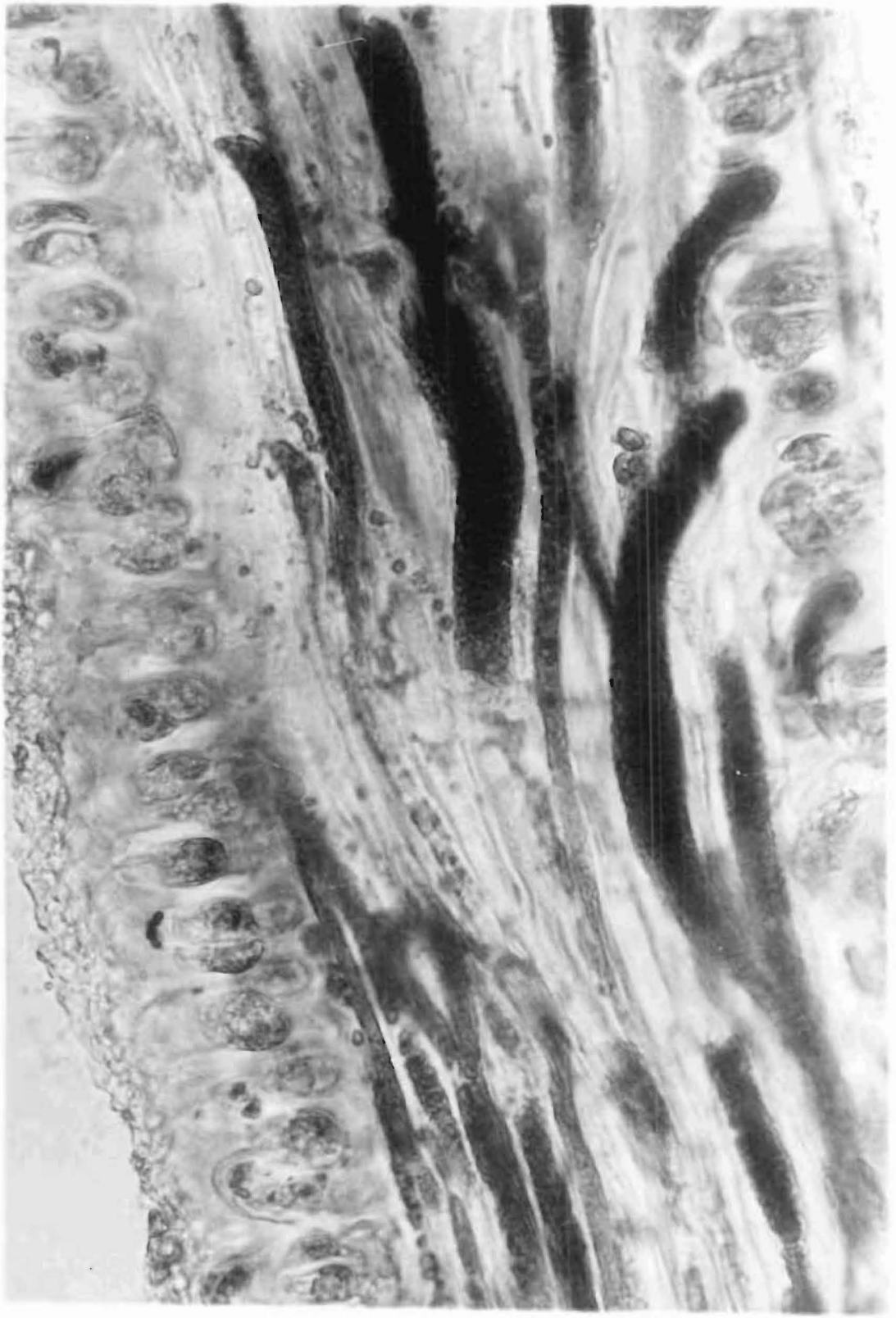
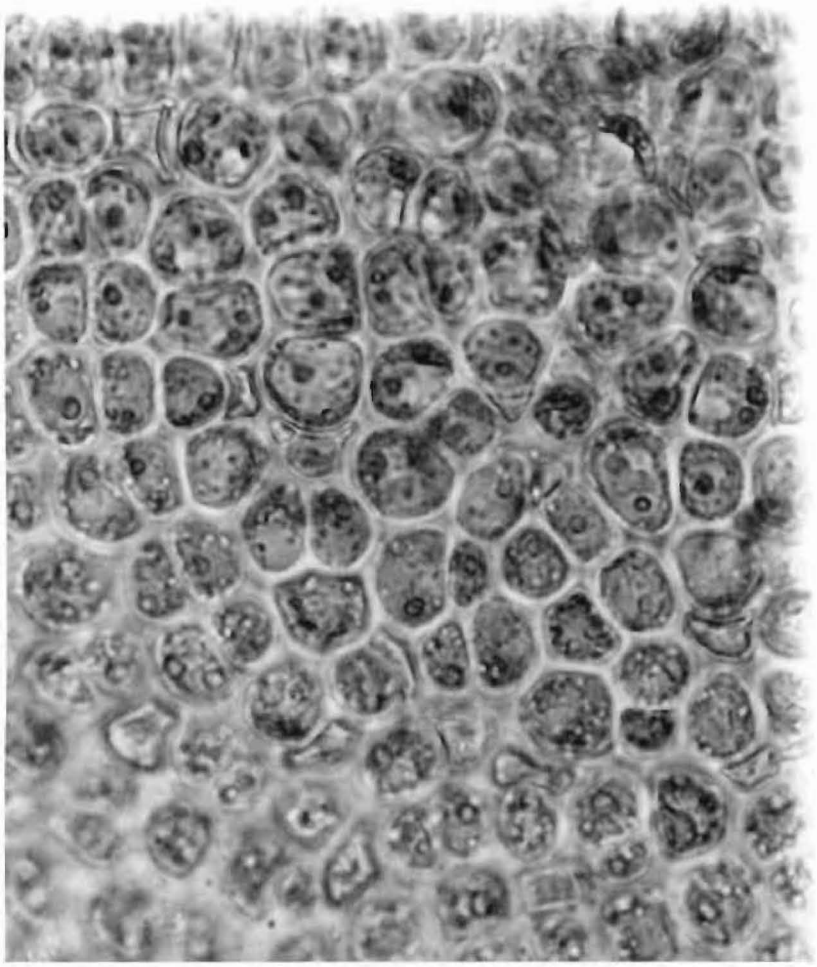


Plate 5.9

Surface view and transverse section of expanded drift thallus of *Ulva lactuca* from McCormack's Bay.



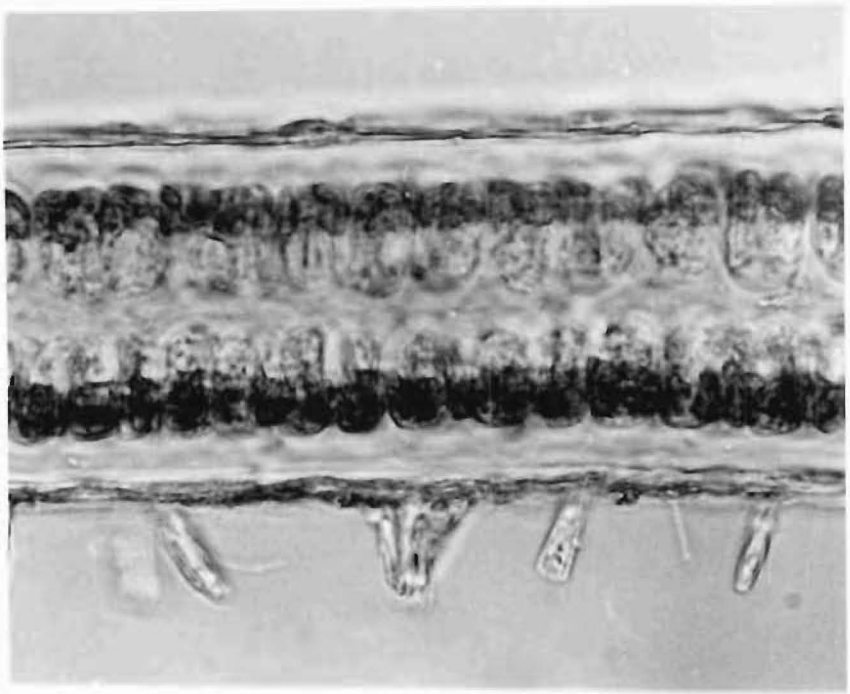
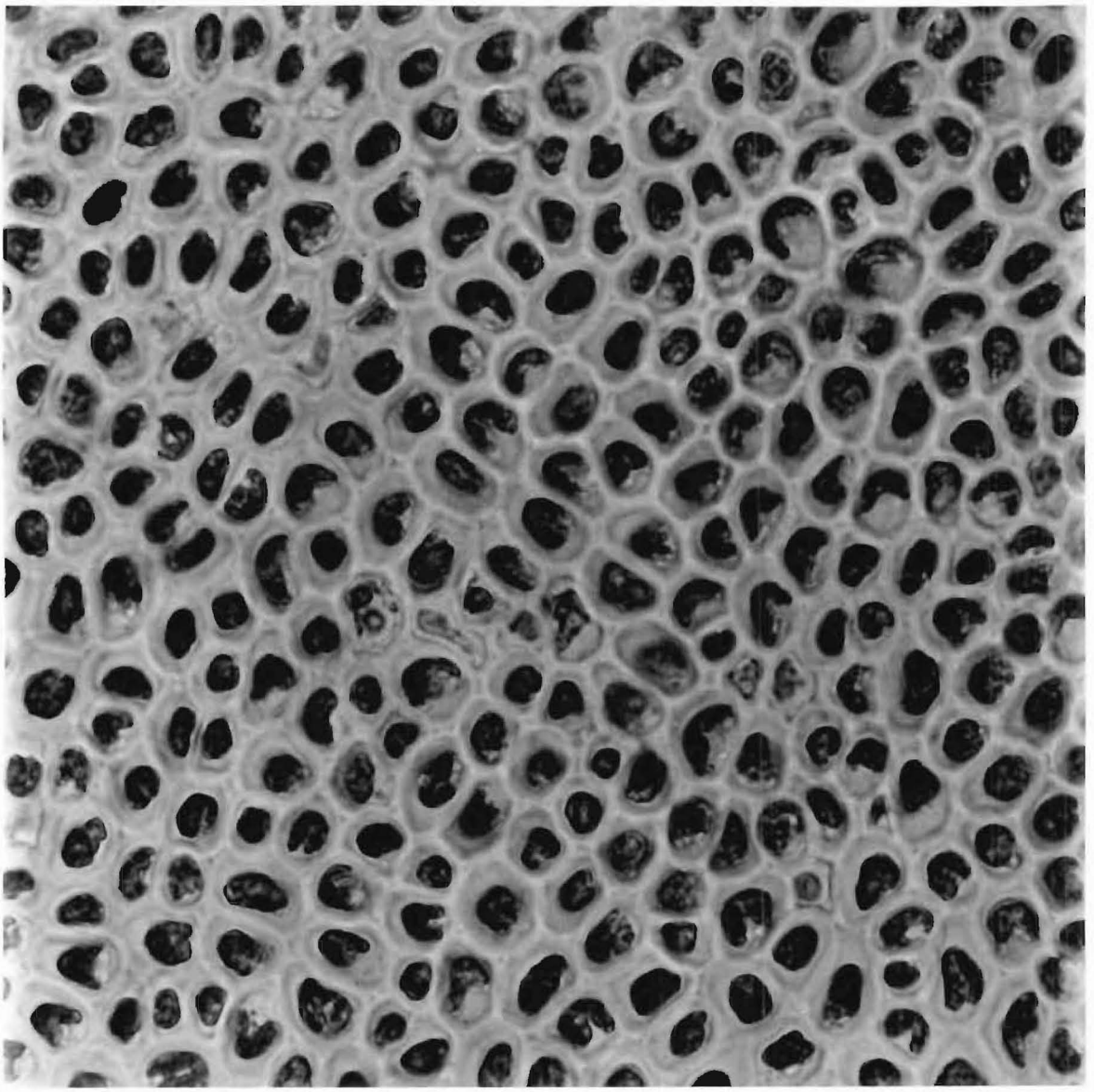


Plate 5.10

Surface view and transverse section of bullate thallus from area C specimen shown on Plate 5.5 (A). Note the contraction of the cell contents.



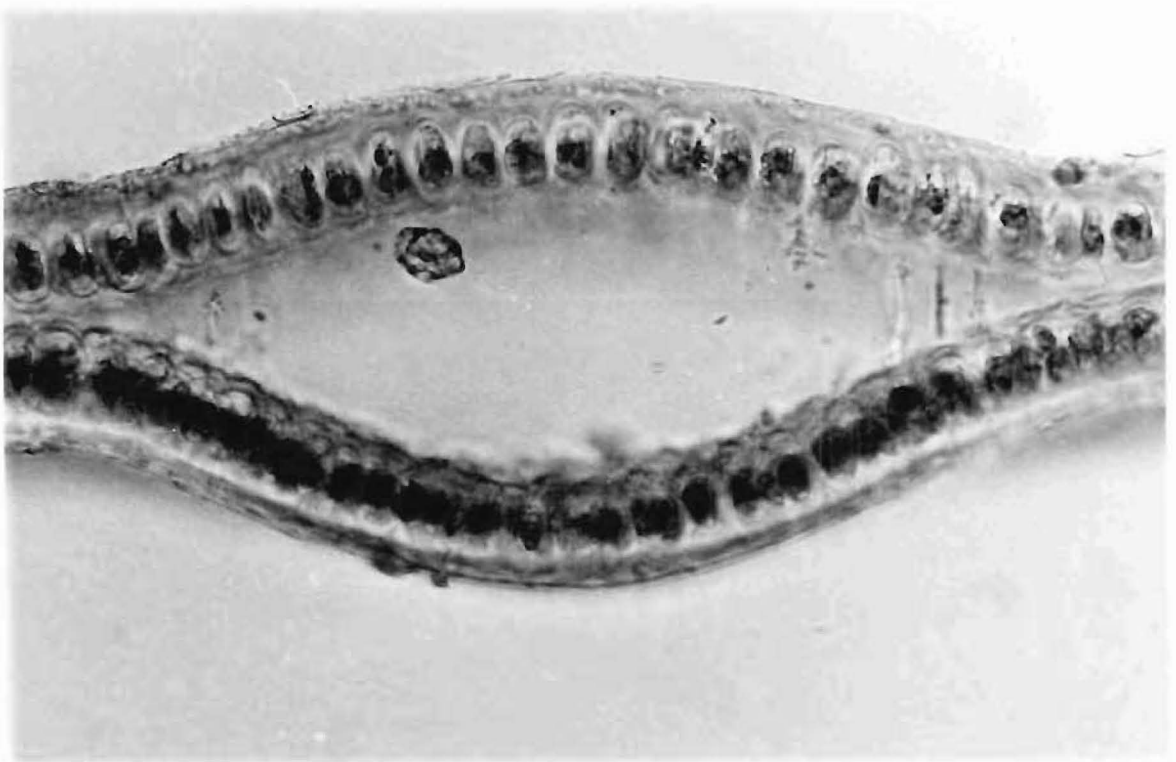


Plate 5.11

Typical appearance of winter form of Enteromorpha ramulosa on the retaining wall, June 1973.

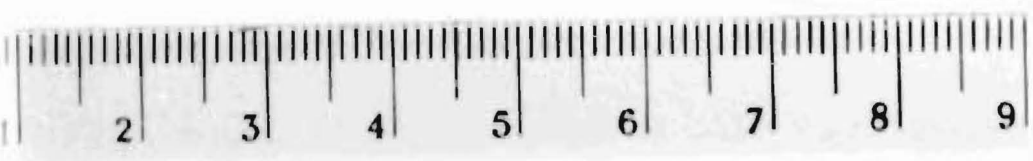

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Plate 5.12

Enteromorpha ramulosa showing a range of size and shape. A and B were attached, C was free floating.

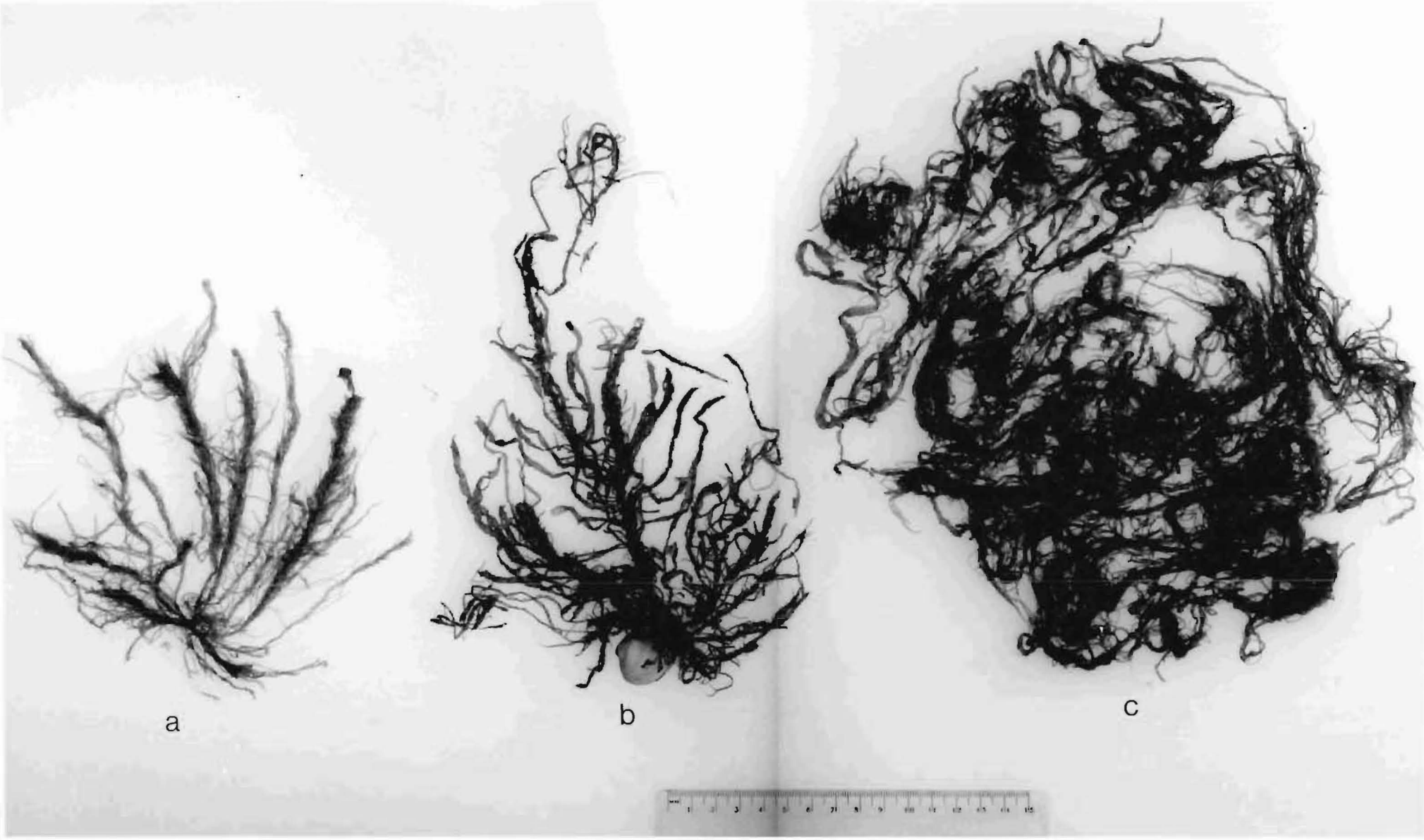
a
b
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Plate 5.13

Example of the upper size limit of attached Enteromorpha ramulosa, January 1972.

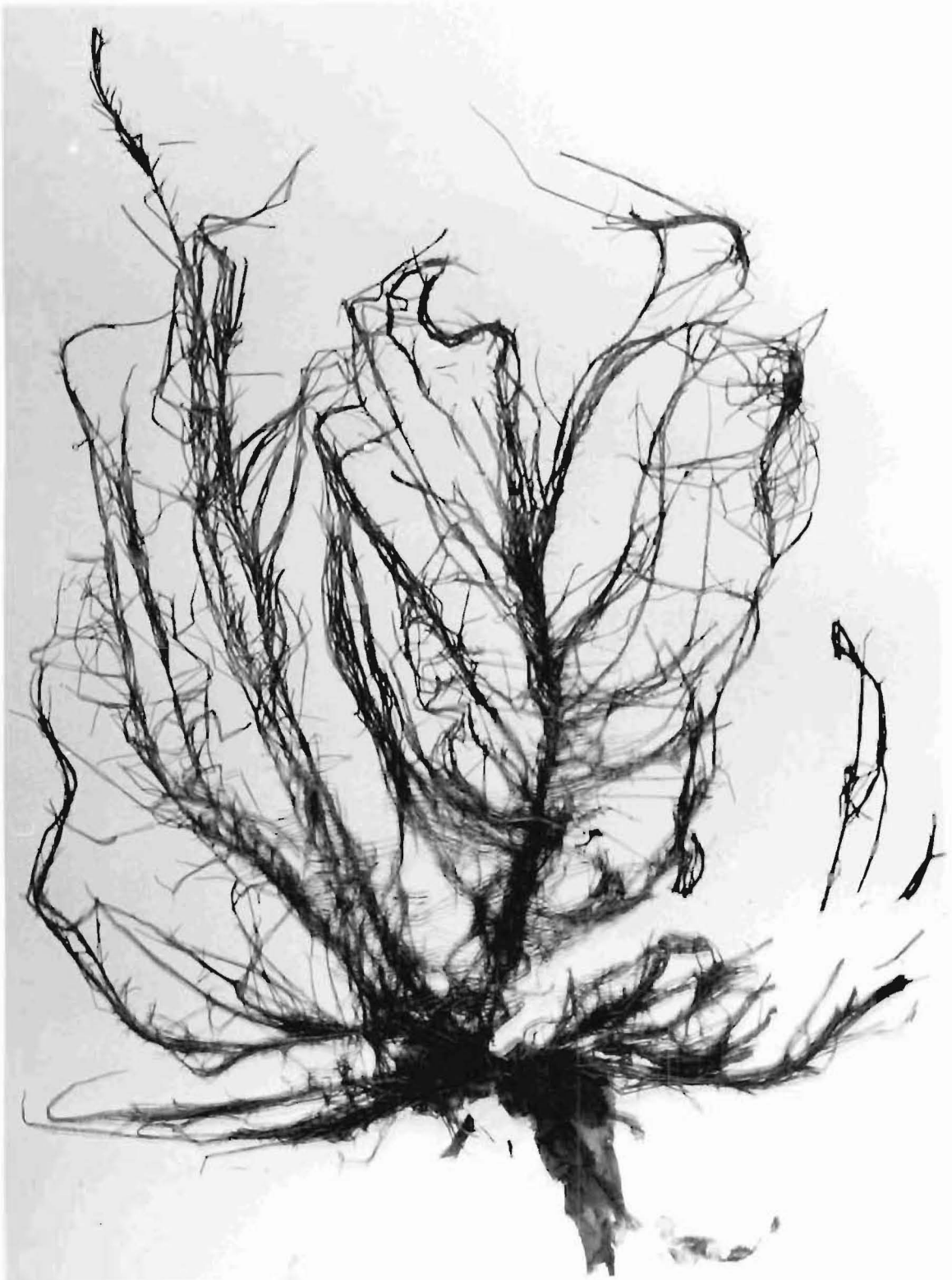


Plate 5.14

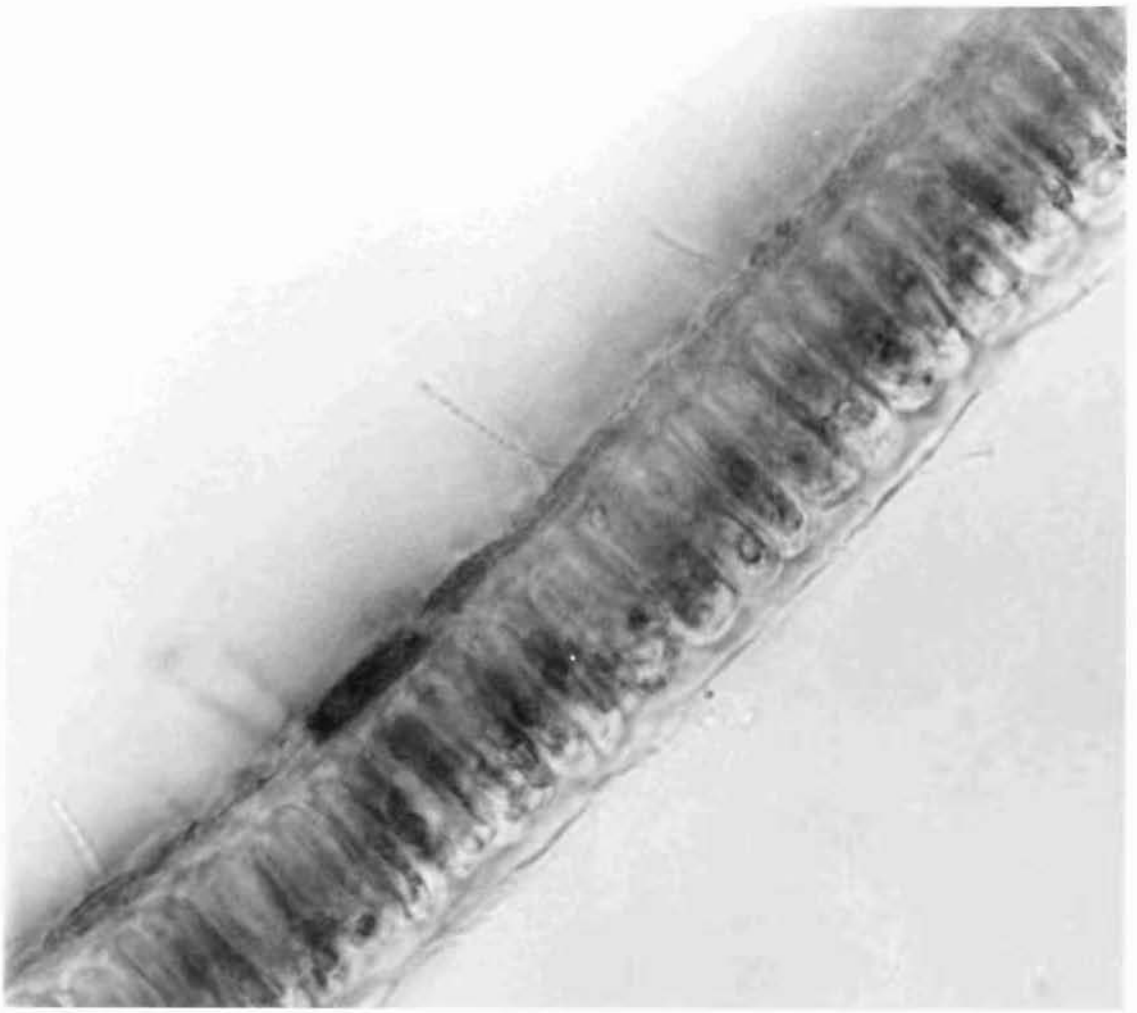
Surface view of branch from specimen A Plate 5.12 showing size and arrangement of cells.

Plate 5.15

Enteromorpha ramulosa showing cells in T.S.

A from specimen A Plate 5.12

B from specimen B Plate 5.12.



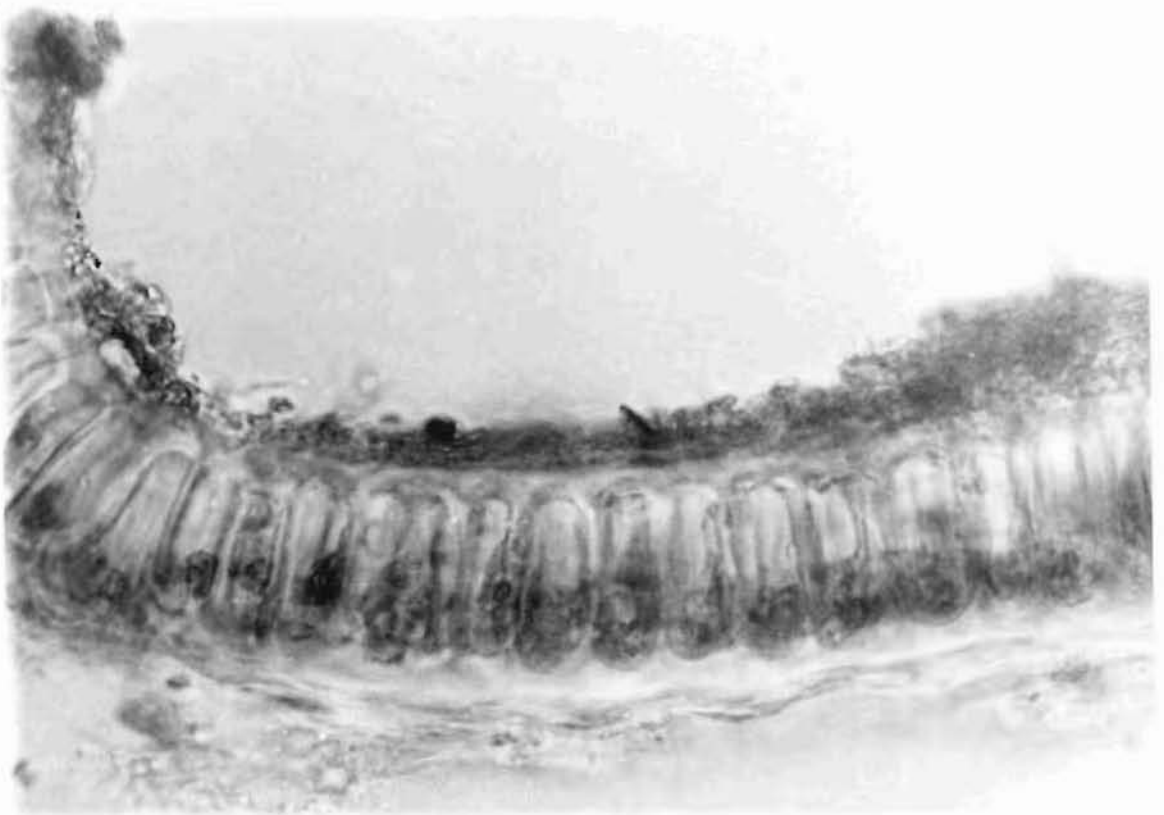


Plate 5.16

Tangled mass of *Chaetomorpha linum* from McCormack's Bay.

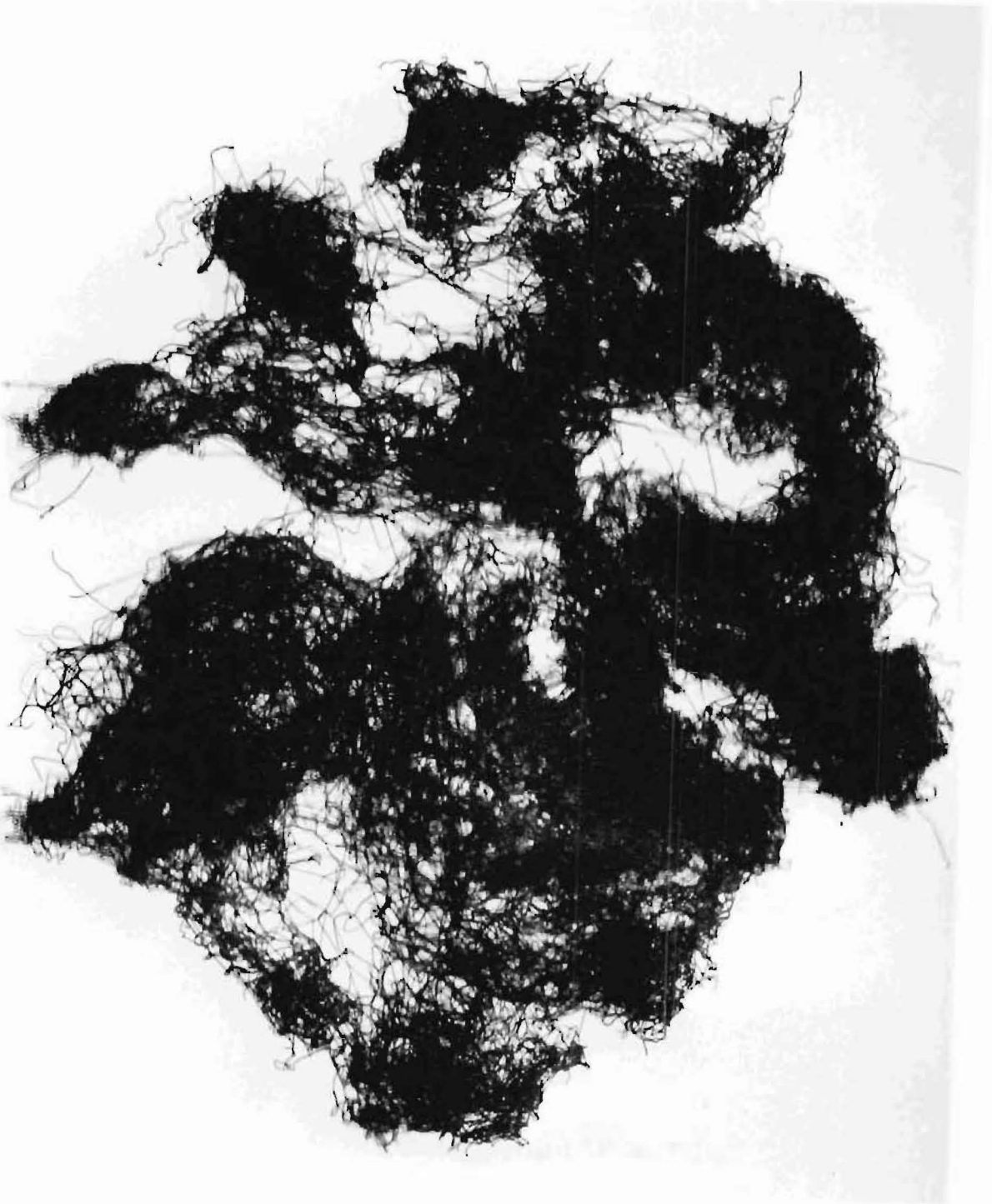


Plate 5.17

Sterile Gracilaria secundata from area C, January 1973.

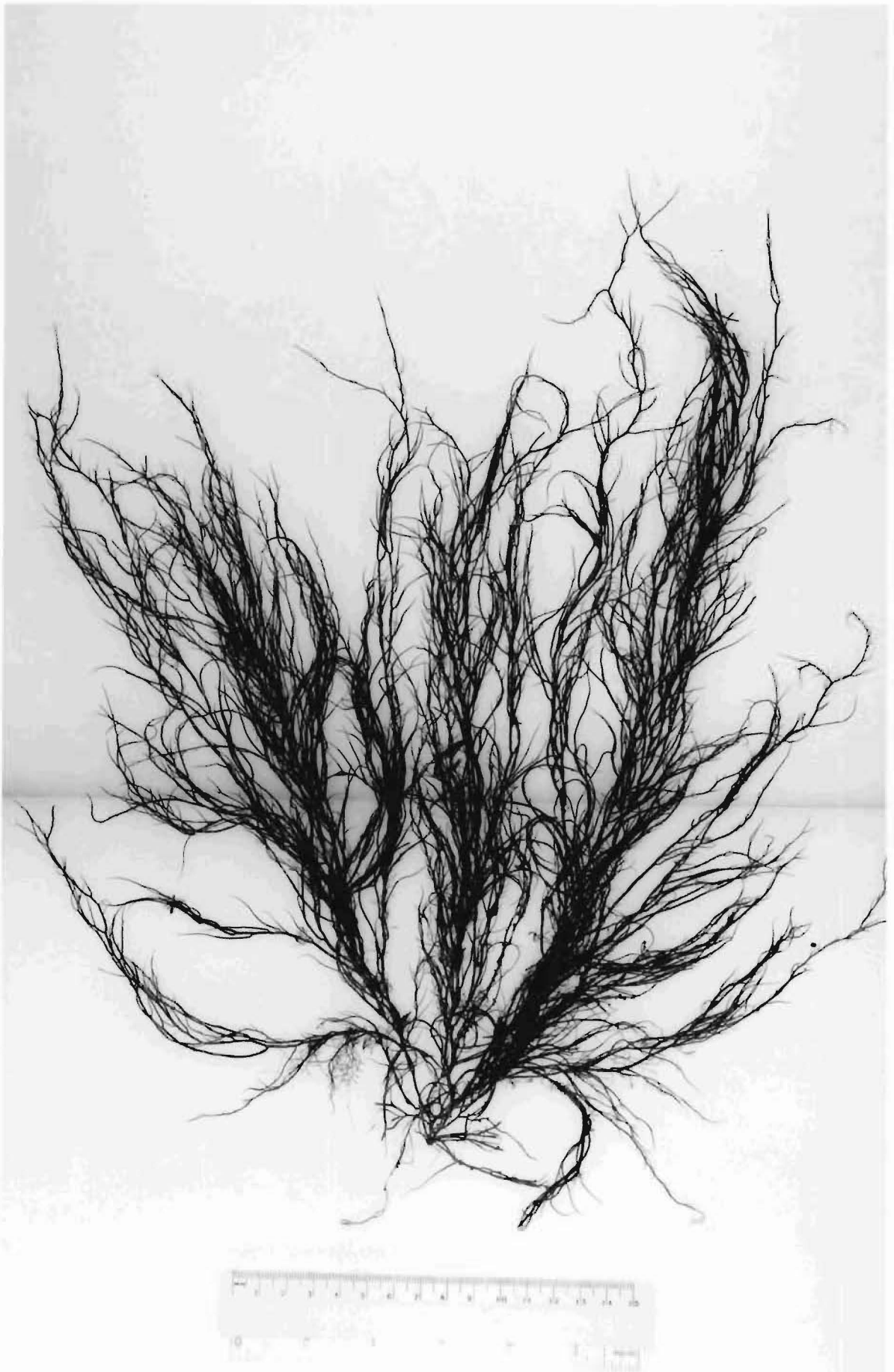
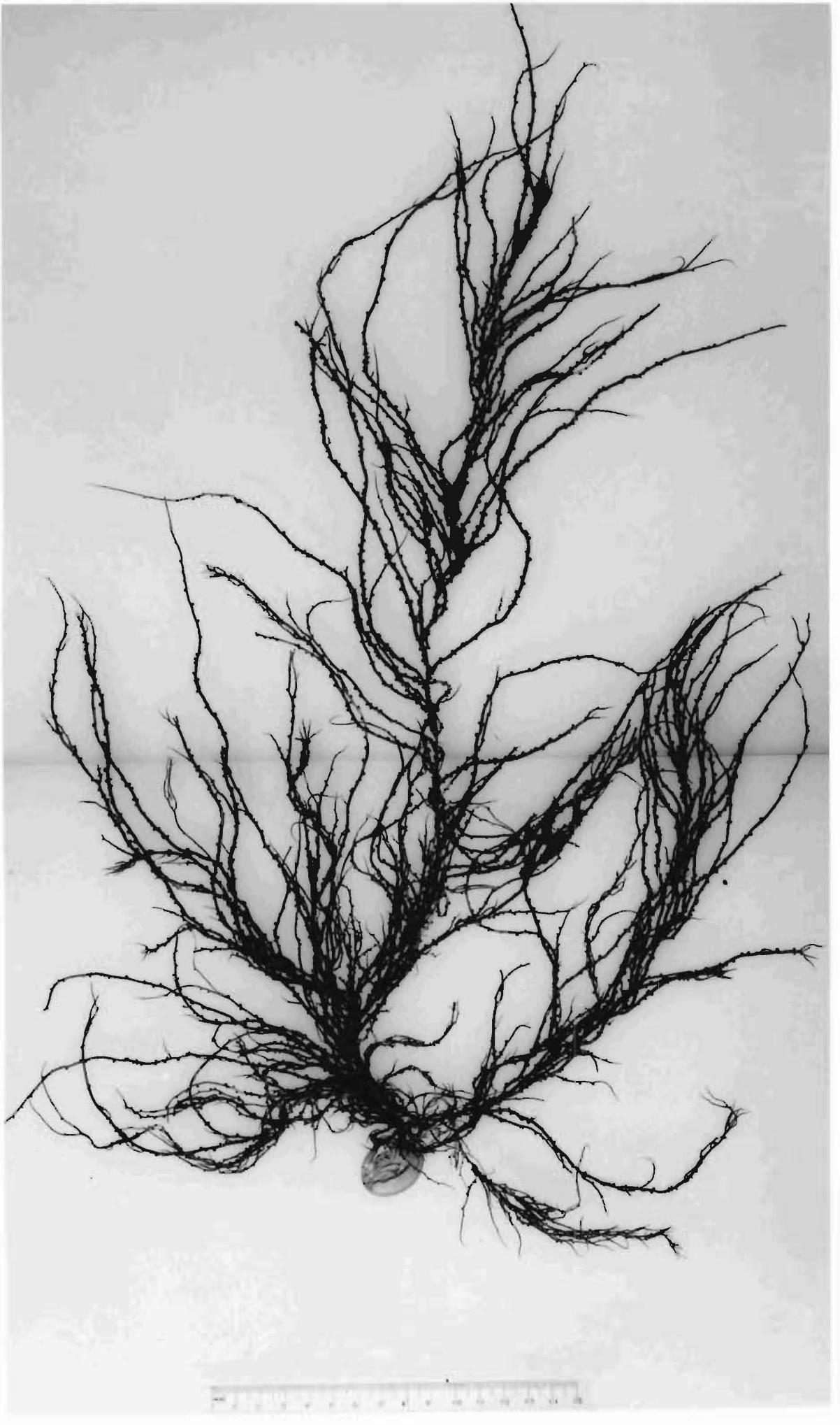


Plate 5.18

Cystocarpic *Gracilaria secundata* from area E, March 1973.



CHAPTER VI

QUANTITATIVE RESULTS OF FIELD STUDY

The results fall into two broad categories: 1) the biota and 2) the environmental variables. The biota will be dealt with under three headings:

(a) The overall distribution as shown from the surveyed and aerial maps.

(b) The per cent cover data from the transects.

(c) The density in dry weight per square metre from the harvested quadrats.

A distinction is made between attached and drift algae which differ in their mode of growth and are affected by different environmental factors. As Ulva lactuca is the most important species, it will be treated in greatest detail. The results of the experimental field work are presented at the end of the chapter.

1) BIOTA

(a) Distribution over the Estuary as a whole (Figs 6.1-6.10)

In May 1971 (Fig. 6.1) the algae were concentrated in the western half of the Estuary, with the main patches of drift algae near the shore off the Bromley Oxidation Ponds (Area D) and Humphrey's Drive (Area C). Smaller patches of

drift occurred in depressions adjacent to the channels of the Avon and Heathcote Rivers with lower concentrations of attached plants adjacent to the drift algae and along the tributory channels. The area covered by the patches of drift and attached algae decreased during the winter (Figs 6.2-6.5), but returned to the May level in November. The mass of drift at Area C was further north in November than in May, but otherwise the pattern of distribution was very similar.

In March 1972 the pattern was similar to that in November 1971, but in June 1972 there was a dramatic decrease in the area of the drift algae and by October 1972 only a small patch of attached algae remained at Area F (Fig. 6.9).

In February 1973 the attached and drift algae were again present at Areas A, B and F, but C and D remained virtually denuded of algae.

The total areas fitting into the 100% and 75-99% cover categories are summarised in Table 6.1 which gives an indication of the abundance of algae on the Estuary. This is only an approximation as the density of the drifts for 1971 is not known. Both the lack of accuracy of the surveys and the variation in densities in the lower per cent cover categories did not justify a similar treatment for them.

The infrared photographs permitted an accurate survey of the areas of moribund drift algae near the mouth of the Heathcote River as shown on Figures 6.6-6.9. These areas disappeared in the winter of 1972 along with the rest of the drift algae.

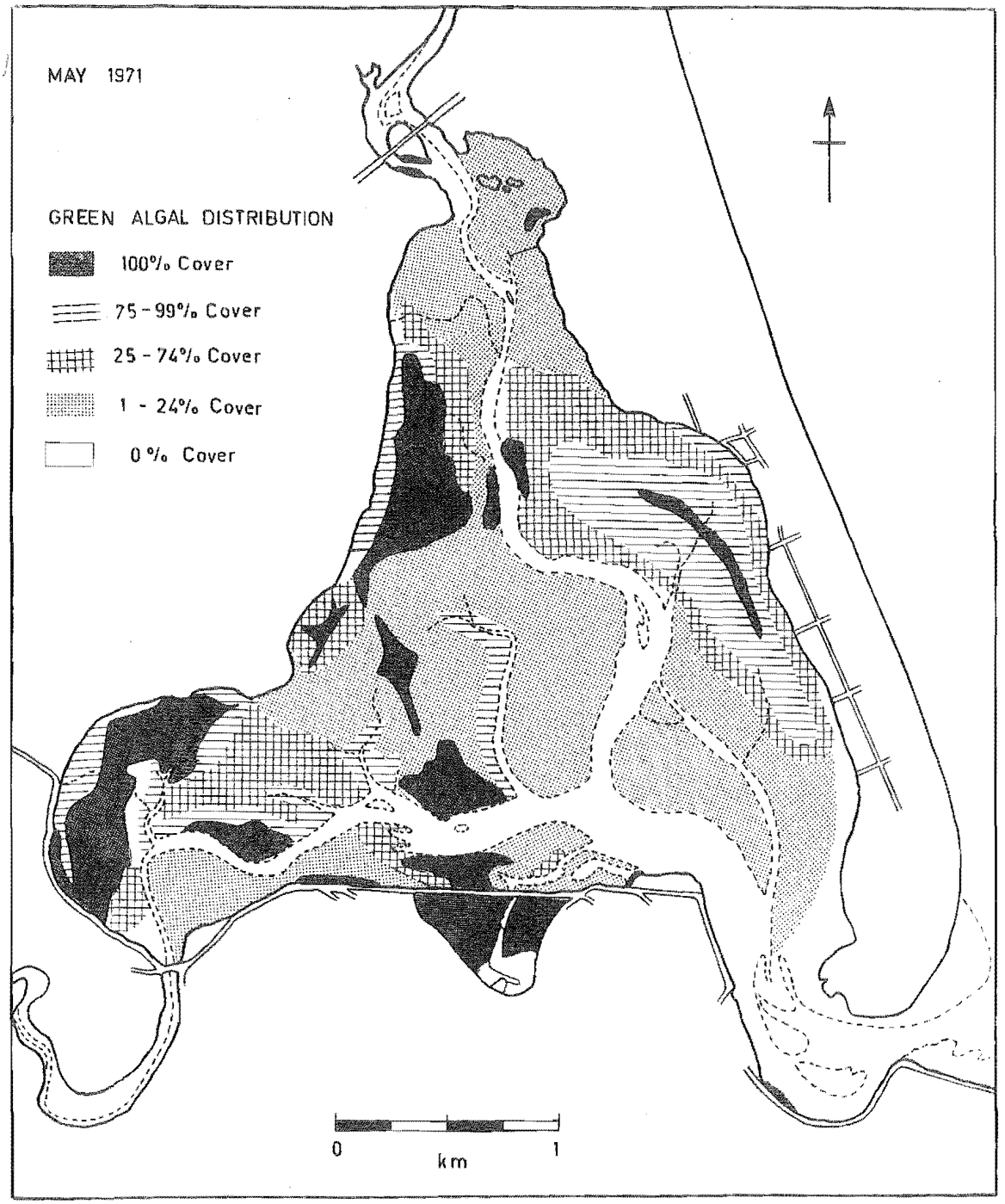


Fig. 6.1 Green algal distribution - May 1971

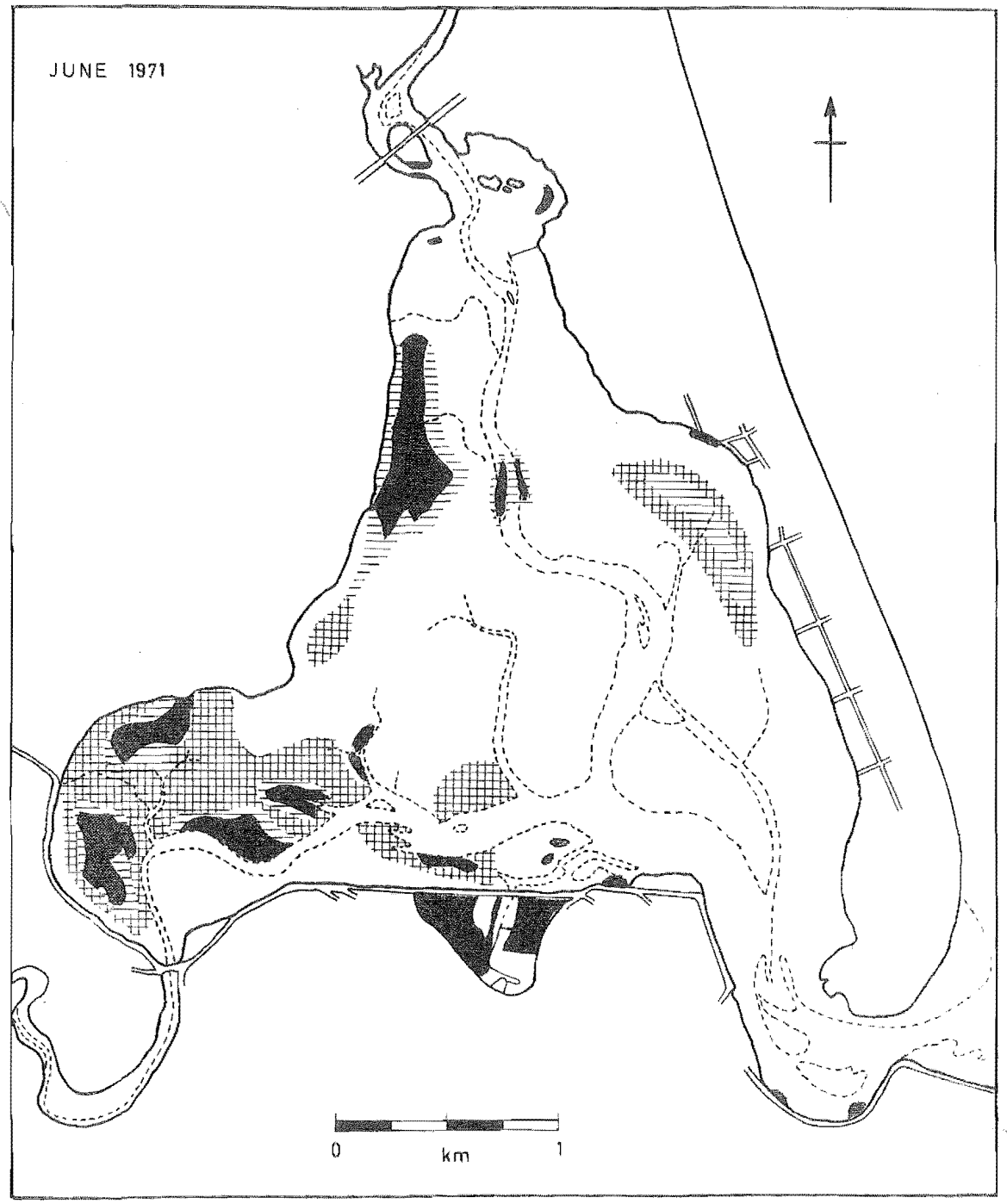


Fig. 6.2 Green algal distribution - June 1971: see Fig. 6.1 for key.

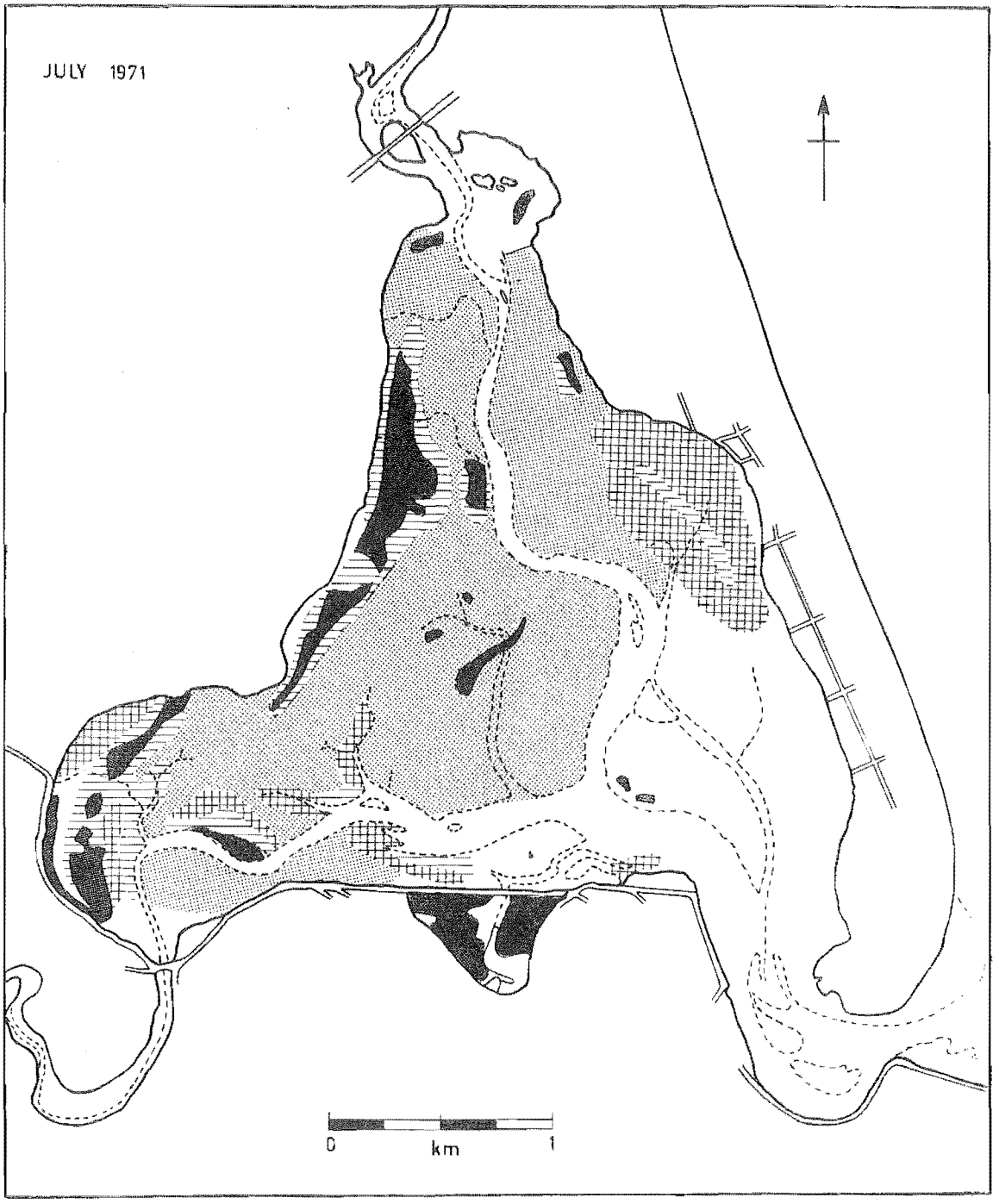


Fig. 6.3 Green algal distribution - July 1971: see Fig. 6.1 for key.

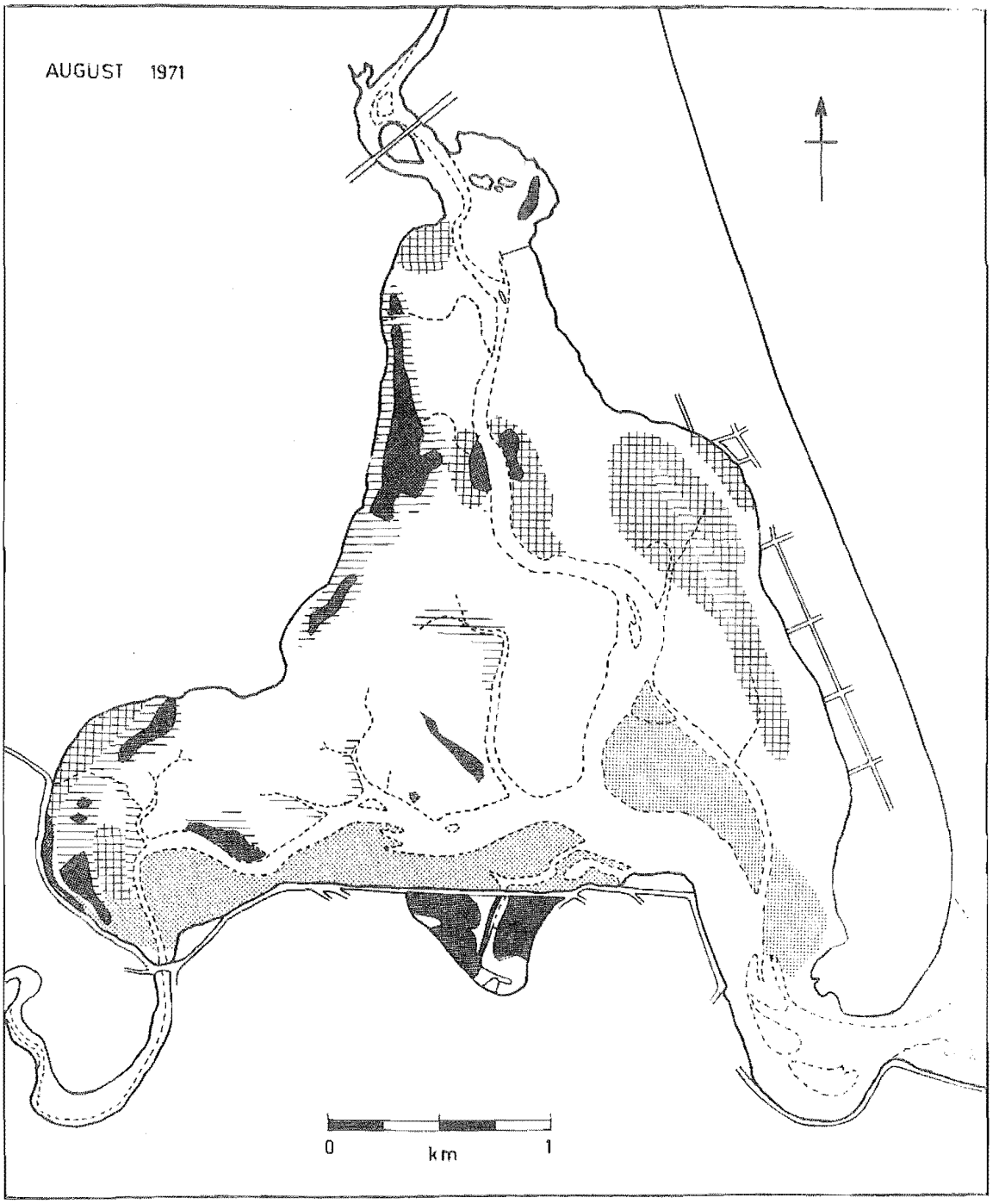


Fig. 6.4 Green algal distribution - August 1971: see Fig. 6.1 for key.

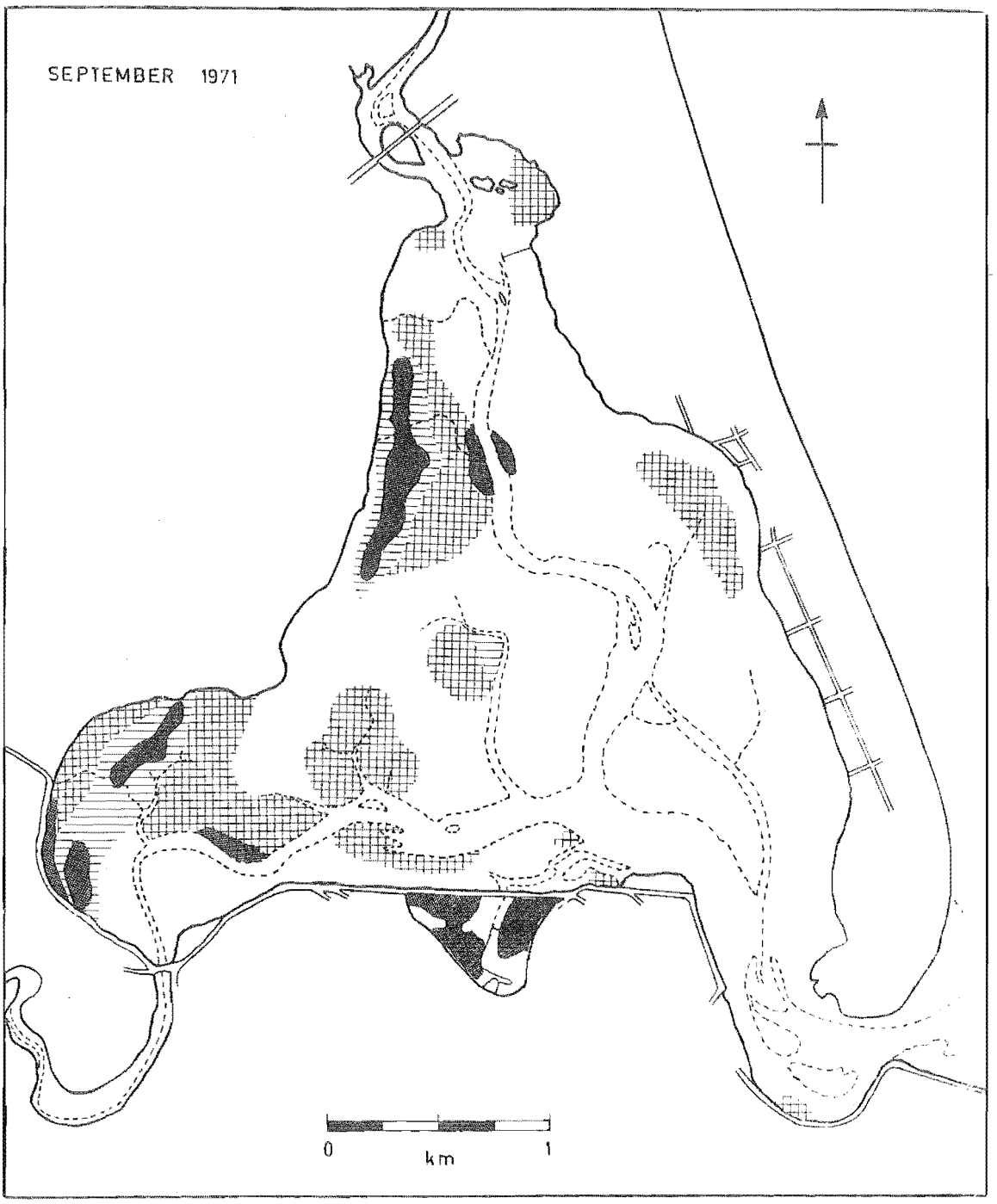


Fig. 6.5 Green algal distribution - September 1971: see Fig. 6.1 for key.

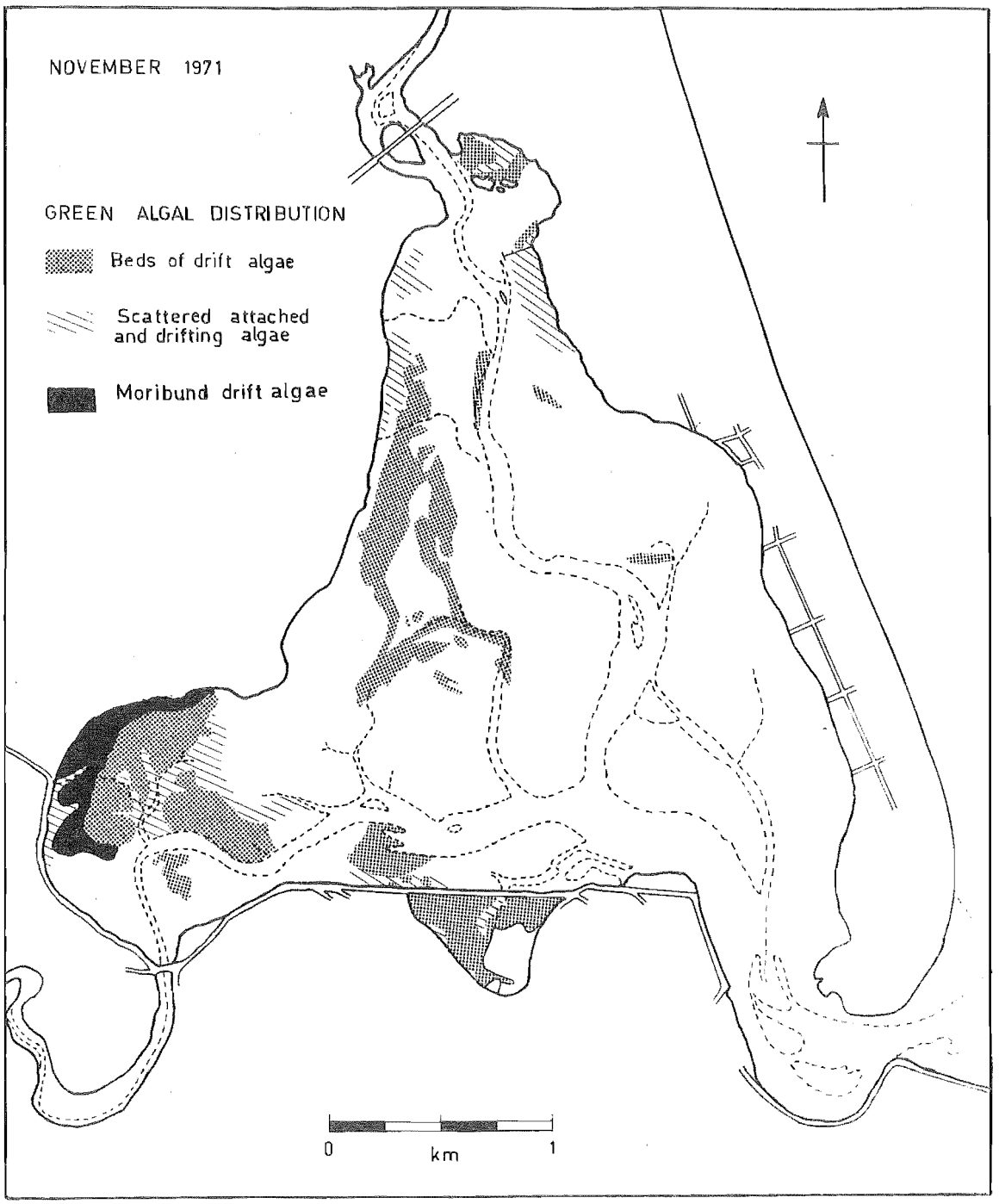


Fig. 6.6. Green algal distribution from aerial photomosaics - November 1971

Fig. 6.7 Green algal distribution - March 1972: see Fig. 6.6 for key.

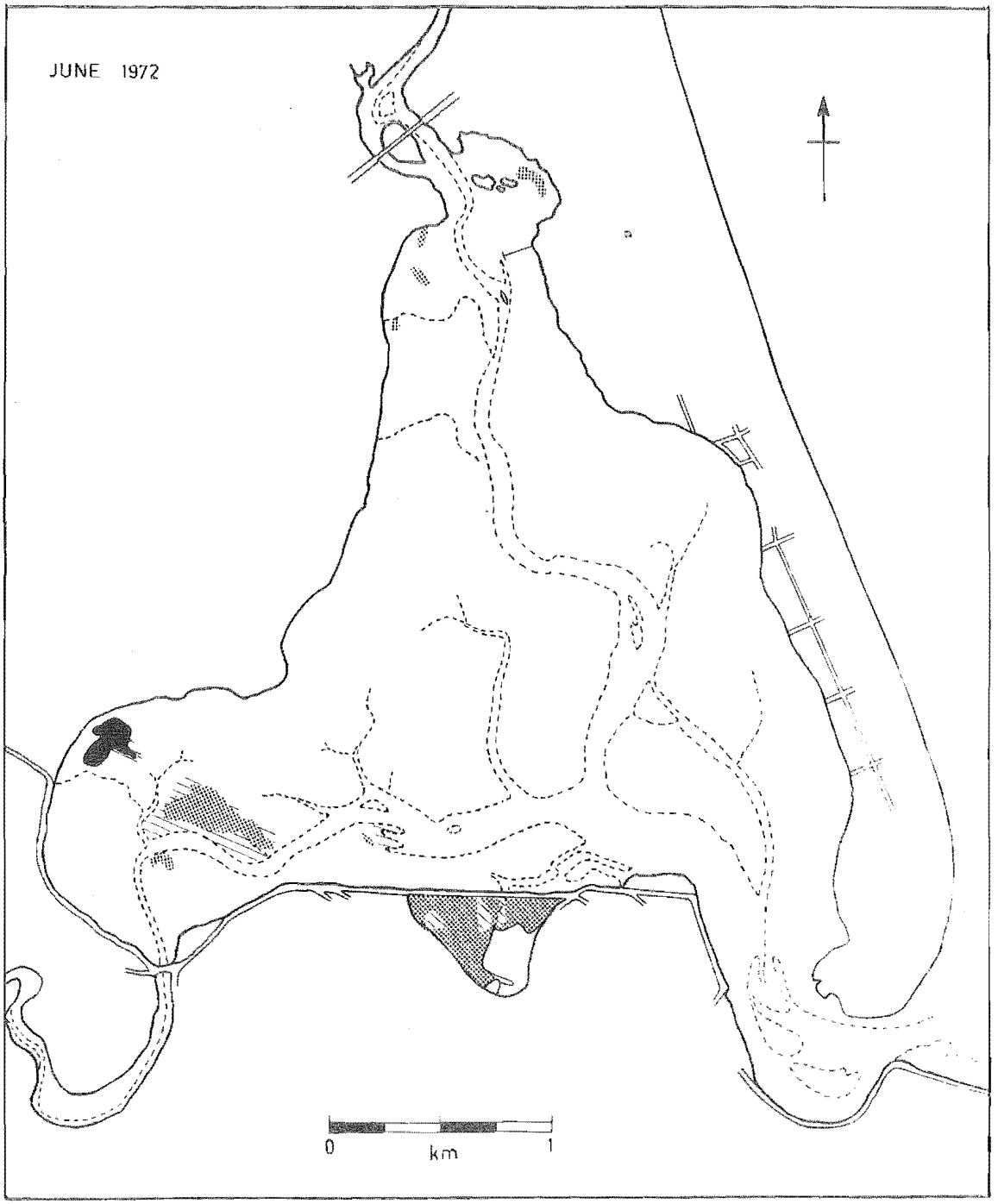


Fig. 6.8. Green algal distribution - June 1972: see Fig. 6.6 for key.

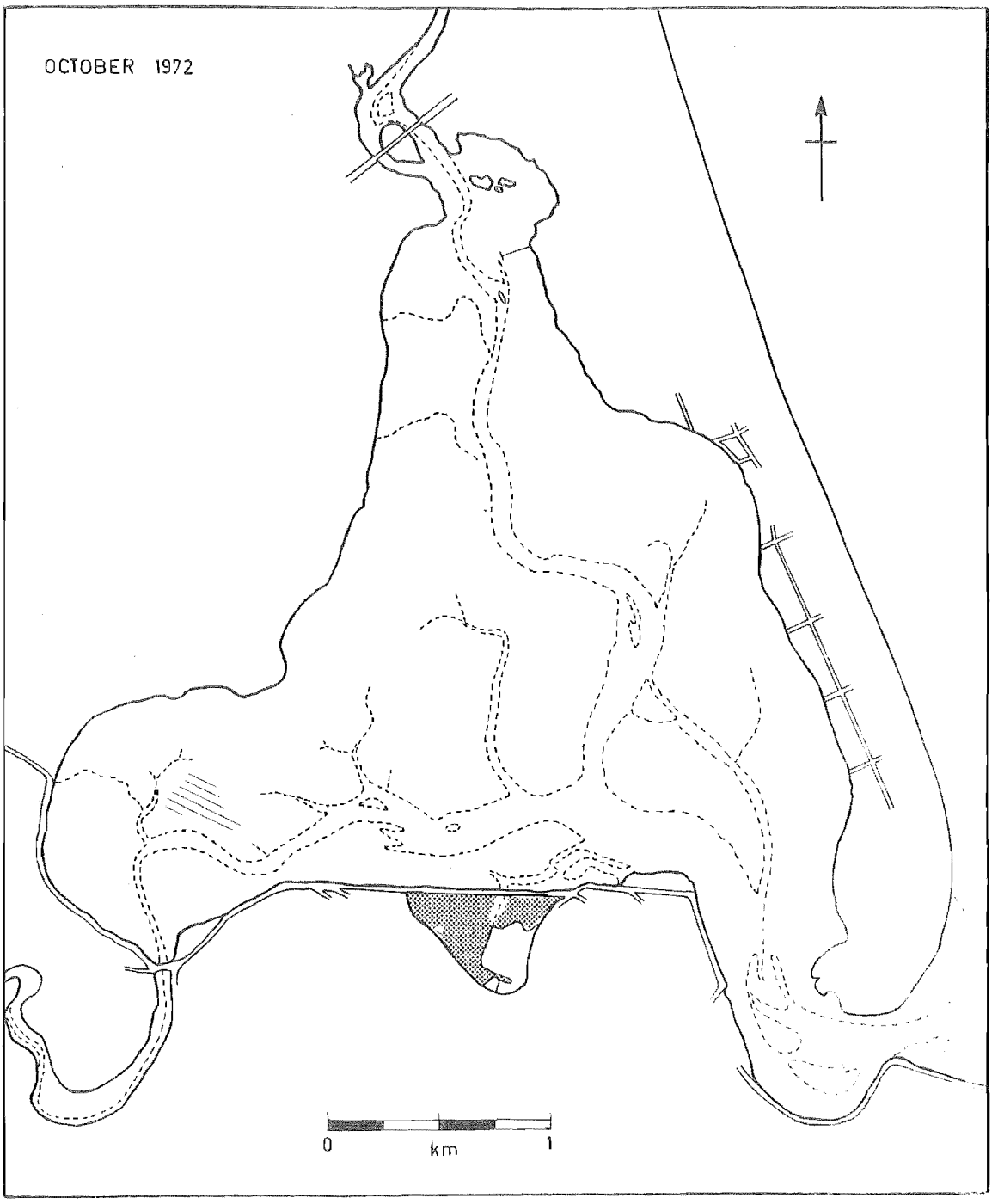


Fig. 6.9 Green algal distribution - October 1972: see Fig. 6.6 for key.

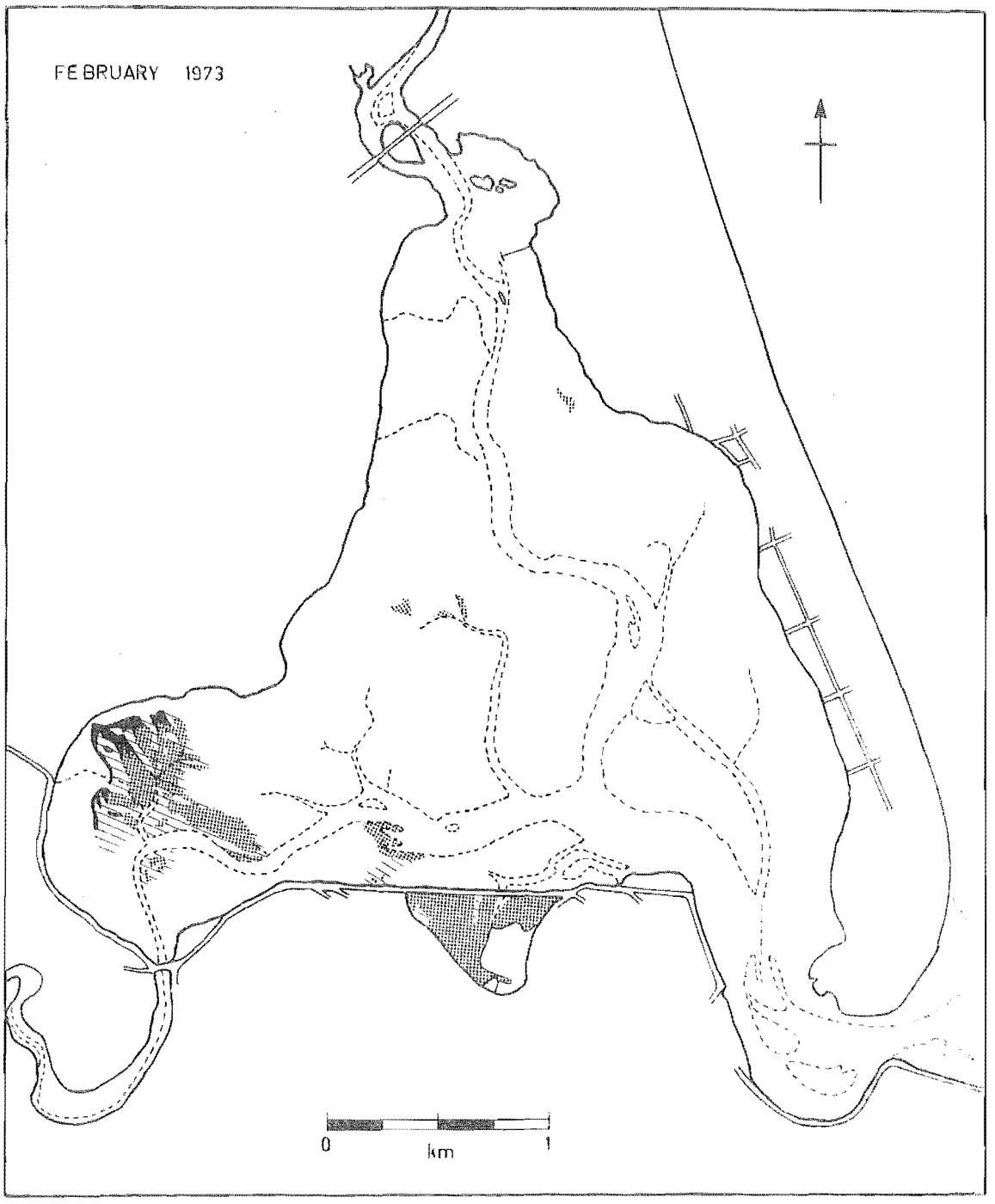


Fig. 6.10 Green algal distribution - February 1973 : see Fig. 6.6 for key.

Table 6.1 Overall per cent cover of Ulva May 1971 to February 1973.

Area of 100% and 79-99% cover in m^2

Month	100% cover	75-99% cover	Moribund algae
May 1971	785,400	1,189,000	-
June 1971	472,000	290,000	-
July 1971	464,000	410,000	-
August 1971	244,000	490,000	-
September 1971	227,000	328,000	-
November 1971	813,000	309,000	113,820
March 1972	528,450	187,000	65,000
June 1972	122,000	65,000	32,000
October 1972	0.0	56,900	0
February 1973	211,400	143,000	0

(b) Per cent cover from transects I-VI May 1971 to January 1972

Of the six transects, I, II and V cut across areas of attached algae, while III and IV were in areas of drift algae. There was never a significant amount of algae along transect VI or in the general area at the southern end of Brighton Spit (see Plate 6.9).

Overall per cent cover of Ulva: The mean per cent cover over all the transects, as summarised in Table 6.2, showed a steady decrease from May to August, dropped sharply in October, but increased in December and January. This overall trend is largely the result of changes at transects III and IV, due to the considerably higher per cent cover at these sites. These results bear out the inadequacy of per cent cover as a measure of density in areas of drift algae. Over transect IV for example per cent cover increased during

the winter, whereas the results from the overall distribution indicated that the area of drift in this region decreased during this period. This anomaly appears to be due to a change in position of the main patch of drift Ulva, resulting in a larger part of the drift lying across the transect. Seasonal changes in standing crop are therefore more accurately reflected by the attached plants at transects I, II and III since, unlike the drift plants, they do not shift with the currents.

At transects I, II and III the attached plants disappeared in June at transect I and in October at transects II and V. They returned to transect I in October and to transect V in January, but transect II remained bare.

Distribution of Ulva along the transects: The attached plants tended to concentrate in the mid to low tide regions, but were limited to areas with solid substrates. The distribution at transect V as shown on Fig. 6.11 is typical of the attached plants as a whole.

The drift plants followed the same general pattern along the transects as the attached plants, but were more variable and tended to concentrate in depressions and pools on the mud flats. A typical distribution pattern is shown for transect III on Fig. 6.11. All the transects had a small patch of drift at the high tide mark, but algae in these patches were in various stages of decomposition. At transect III the main patch of drift extended further towards the high-tide mark than at the other transects, particularly in winter. The thalli in the upper part of the transect were predominantly of the bullate type.

Table 6.2 Per cent *Ulva* cover on transects I to V between May 1971 and January 1972.

		I	II	III	IV	V	Totals	Mean % cover
May	1971	1.3	4.4	67.0	44.0	20.0	126.7	25.4
June	"	0.7	6.6	48.8	50.0	11.2	117.3	23.5
July	"	0	0	33.0	68.5	10.2	111.7	22.2
Aug.	"	0	3.3	27.6	61.8	10.2	102.9	20.6
Sept.	"	0	3.3	12.5	54.6	3.5	73.9	14.8
Oct.	"	14.3	0	27.9	54.0	0	71.6	14.3
Nov.	"	15.0	0	22.2	45.0	0	67.2	13.5
Dec.	"	19.0	0	31.4	50.0	0	81.4	16.3
Jan.	1972	17.8	0	58.4	45.0	6.0	108.4	21.9
Totals		68.1	17.6	328.8	472.9	60.6		
Mean %		6.2	2.0	36.5	52.6	6.5		

Table 6.3 Per cent cover of *Enteromorpha* and *Gracilaria* transects III, IV and V between May 1971 and January 1972.

		III		IV		V	
		Entero-morpha	Graci-laria	Entero-morpha	Graci-laria	Entero-morpha	Graci-laria
May	1971	-	-	0.2	0.4	6.4	0.6
June	"	-	2.4	0.4	-	0.4	1.1
July	"	-	2.5	-	-	0.2	0.9
Aug.	"	-	0.6	-	-	0.1	0.6
Sept.	"	-	2.4	-	-	-	0.4
Oct.	"	-	0.4	0.3	-	-	-
Nov.	"	0.25	0.4	0.6	-	-	-
Dec.	"	1.4	0.5	2.8	-	-	-
Jan.	1972	13.0	1.6	4.8	-	1.0	-

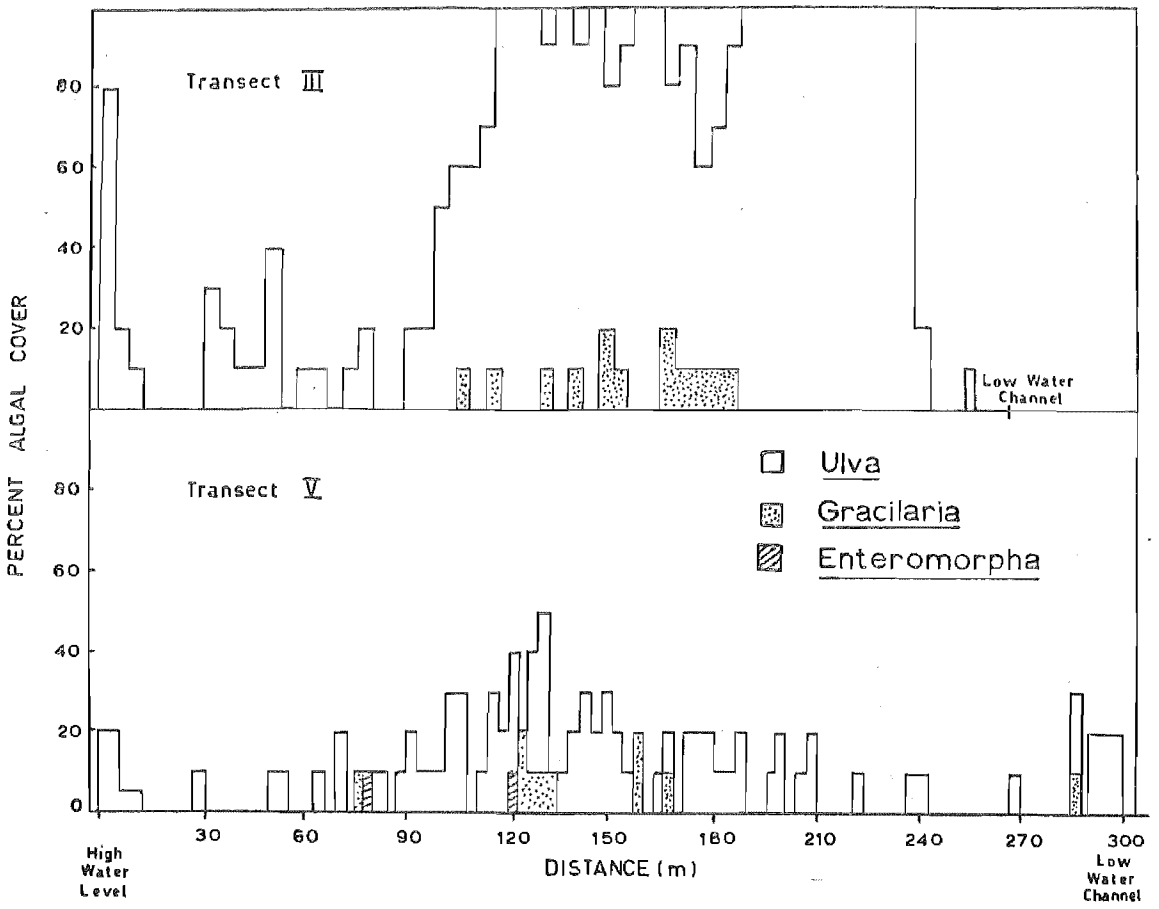


Fig. 6.11 Per cent total area of the transect covered in algae in June 1971.

Transect III: Ulva 48.8%, Gracilaria 2.4%
Transect V: Ulva 10.2%, Gracilaria 0.8%, Enteromorpha 0.2%.

Overall per cent cover of Enteromorpha ramulosa and Gracilaria secundata: These two were of less importance than Ulva and occurred only at transects III, IV and V (Table 6.3).

(c) Density as dry weight per area - January 1972 to May 1973

The large seasonal variations and the considerable differences in standing crop between areas and species are more conveniently shown on a logarithmic scale. This scale is recommended by Williamson (1972), who lists the following advantages:

Firstly, the relative changes in populations are more easily studied.

Secondly, the logarithm of a population is more symmetrically distributed than the population size itself.

Thirdly, it is more convenient for highly variable populations.

Fourthly, many forms of growth are exponential and autocatalytic in nature. Growth curves of this type form a straight line on a logarithmic scale. The confidence limits shown on Figs 6.13 and 6.14 were calculated using the formula

$$\bar{Y} - \frac{S}{N} t\ 95\% \leq u \leq \bar{Y} + \frac{S}{N} t\ 95\%$$

(Snedecor and Cochrane, 1967)

Seasonal Variation: The monthly grand totals from the 37 original sampling sites (at areas A, B, C, D and E) are shown on Fig. 6.12. Area F is excluded as it was not sampled until November 1972. Aa refers to attached algae and Ad refers to drift algae. Ulva lactuca was consistenly the most abundant and widespread alga during this study, while Enteromorpha ramulosa and Gracilaria secundata were less common and more variable. U. lactuca reached its peak density in February 1972 and March 1973 and its minimum density in August 1972. G. secundata followed a similar pattern, but was absent in June, July and August 1972. E. ramulosa had the greatest seasonal fluctuation, occurring only from November to March, with a rapid increase to a peak density in February and an equally rapid decline.

Ulva and Enteromorpha occurred at all sampling sites, but Enteromorpha was abundant only at site B. Gracilaria has a more disjunct distribution and was important at only C and

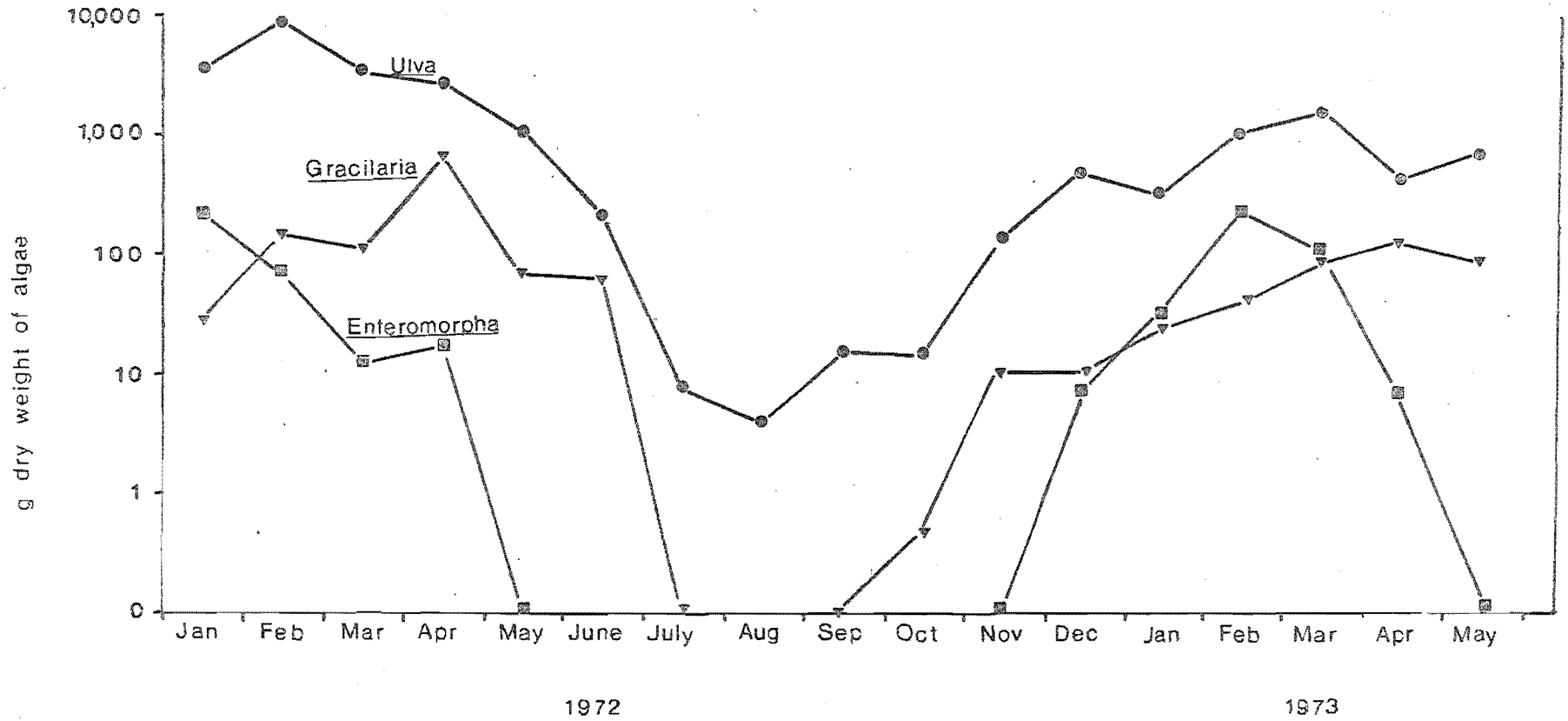


Fig. 6·12 Total weight of algae from all sample sites except area F

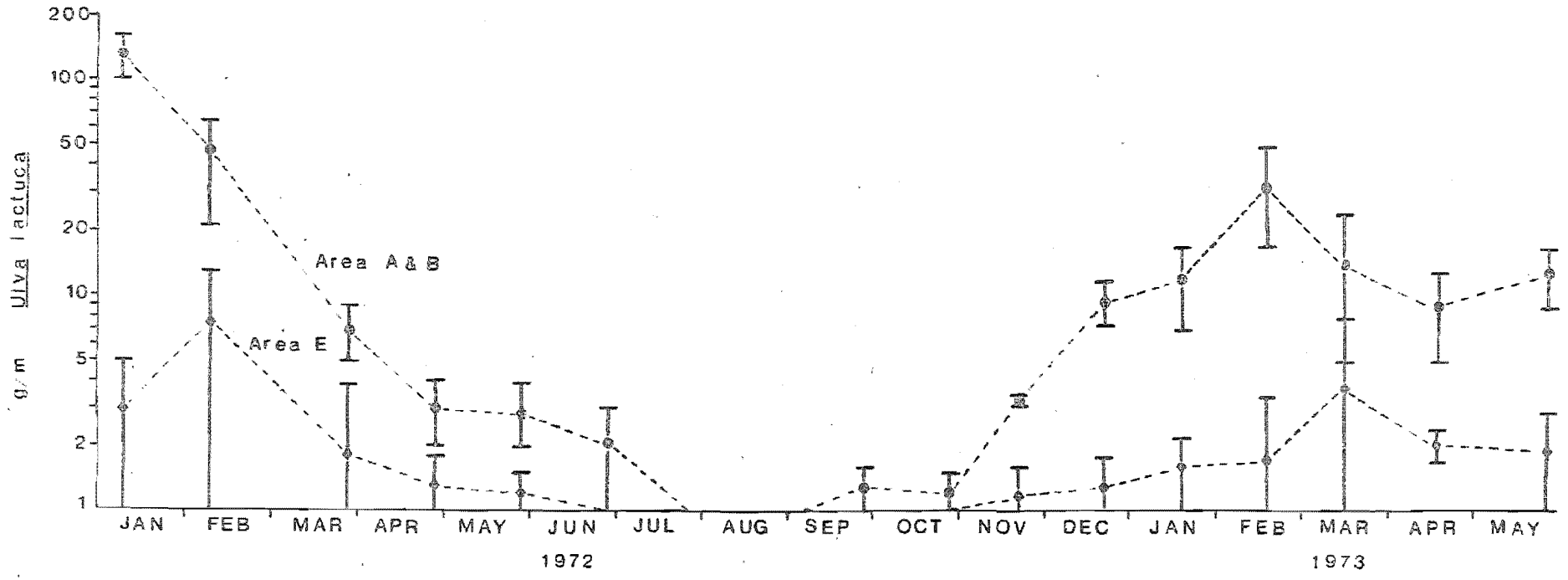


Fig 6·13 Density of attached plants of Ulva lactuca

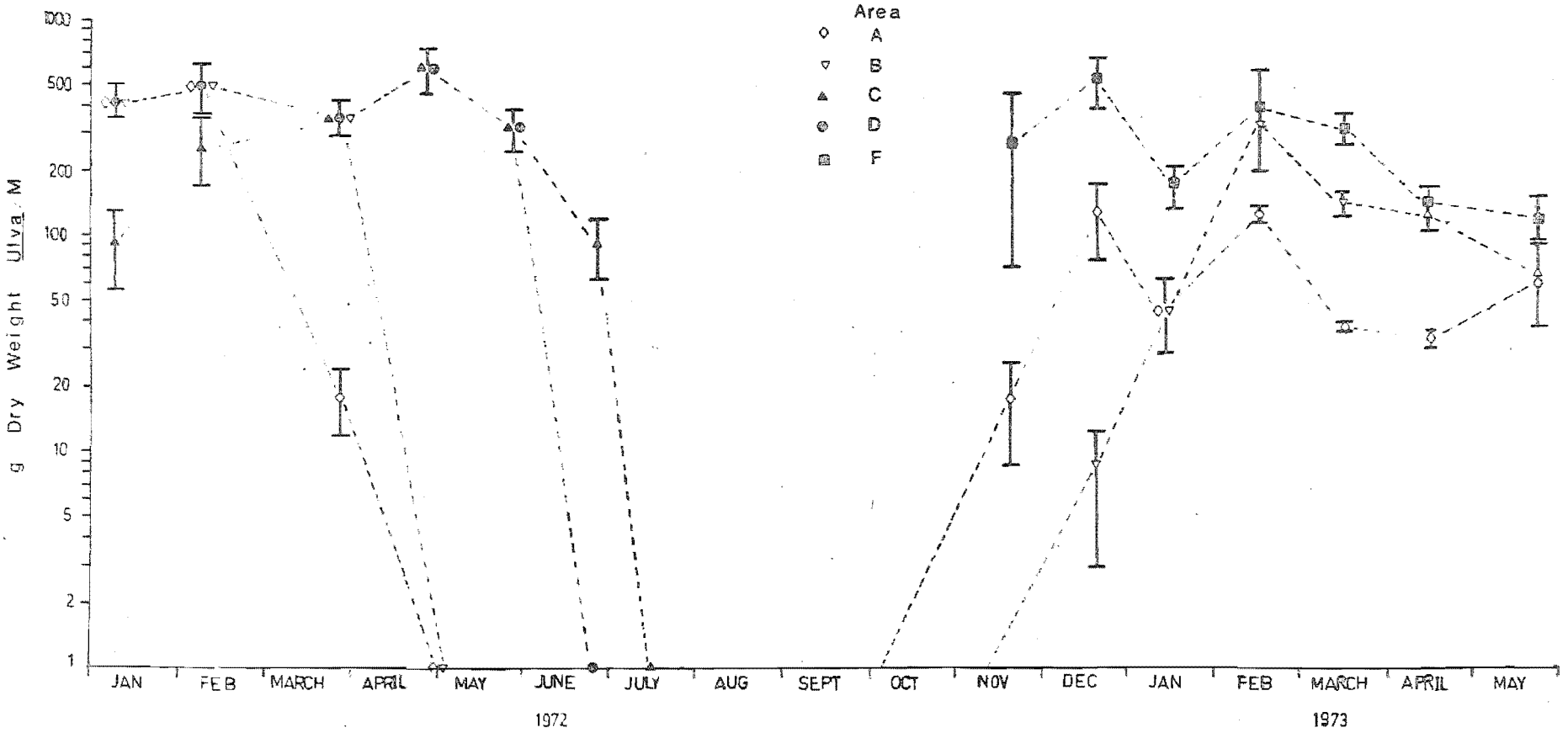


Fig 6·14 Density of Ulva lactuca drift thalli in g/m

quadrat (1).

Distribution of Ulva lactuca: The density of Ulva and the relative proportions of drift and attached plants varied considerably between sites. Attached plants occurred at Aa, Ba and Fa in similar densities and at very much lower densities at E (Fig. 6.13). The seasonal change in density of attached plants followed a similar pattern for all sites. The density decreased evenly from a peak in January 1972 to zero in August 1972 but increased from October 1972 to March 1973, the rate being approximately exponential.

The density of the unattached plants (Fig. 6.14) was generally higher than that of the attached plants and was generally similar for each site except; for C which was significantly lower than A, B and D in January and February 1972 and A, which was significantly lower than the other sites in March 1972. The drift plants had disappeared from sites A and B in April, site D in June and site C in July. This contrasts with 1971 when the drift patches persisted throughout the winter. They reappeared at Fd and Ad in November and at Bd in December 1972. The density at Fd was significantly higher than at Ad and, with the exception of April, was also significantly higher than Bd. The density at B was lower than Ad in December, equal to it in January, intermediate between Ad and Fd in February and similar to Fd in April.

As both density of, and the area covered by the algae will vary, neither, by itself, gives an accurate estimation of the total standing crop. By multiplying the density of the algae by the area they covered, a more accurate estimate can be gained as shown on Table 6.4 (see Fig. 6.14 for densities). October 1972 is excluded as virtually no algae were detected

Table 6.4 Standing crop from sample areas in kg dry weight

	Area	March 1972	June 1972	February 1973
Drift algae	A	16,302	12	3,220
	B	5,000	6,000	3,470
	C	49,000	0	0
	D	109,000	0	0
	Total	179,302	6,012	6,690
Attached algae	A	1,465	0	2,076
	B	96	92	342
	Total	1,561	92	2,418
Grand Total		180,863	6,104	9,108

in the aerial surveys.

Area F in February 1973 contained 950 grams of attached algae and 17,000 kilograms of drift.

Table 6.4 highlights the dominance of the drift over attached Ulva in summer. In March 1972 the ratio of drift to attached total standing crop was approximately 120:1. In February 1973 the total drift standing crop was only $\frac{1}{26}$th of that found in March 1972, but was still 2.7 greater than the attached standing crop. This reduction of drift standing in the 1972/73 summer was a result of the disappearance of all drift plants in the winter of 1972. The drift standing crop returned at areas A and B in the 1972/73 summer at levels lower than in 1971/72, but did not return at all to areas C and D. In contrast, to the drift, attached standing crop was 1.5 times greater in February 1973 than in March 1972.

Plate 6.1

Drift Ulva lactuca near the high tide mark at area C, September 1971.

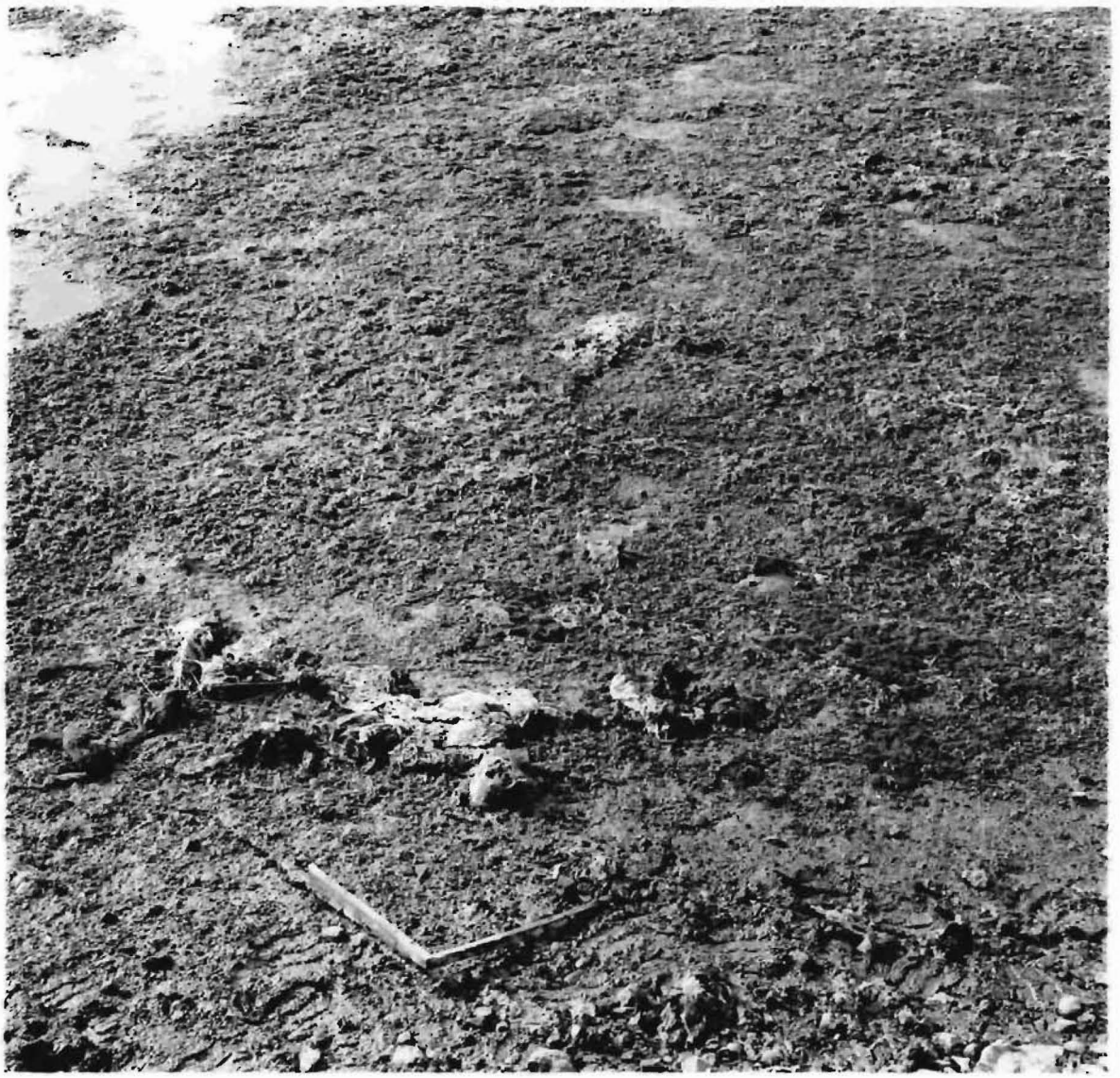


Plate 6.2

Interlaced *Ulva lactuca* and *Enteromorpha* at area A, December 1972.

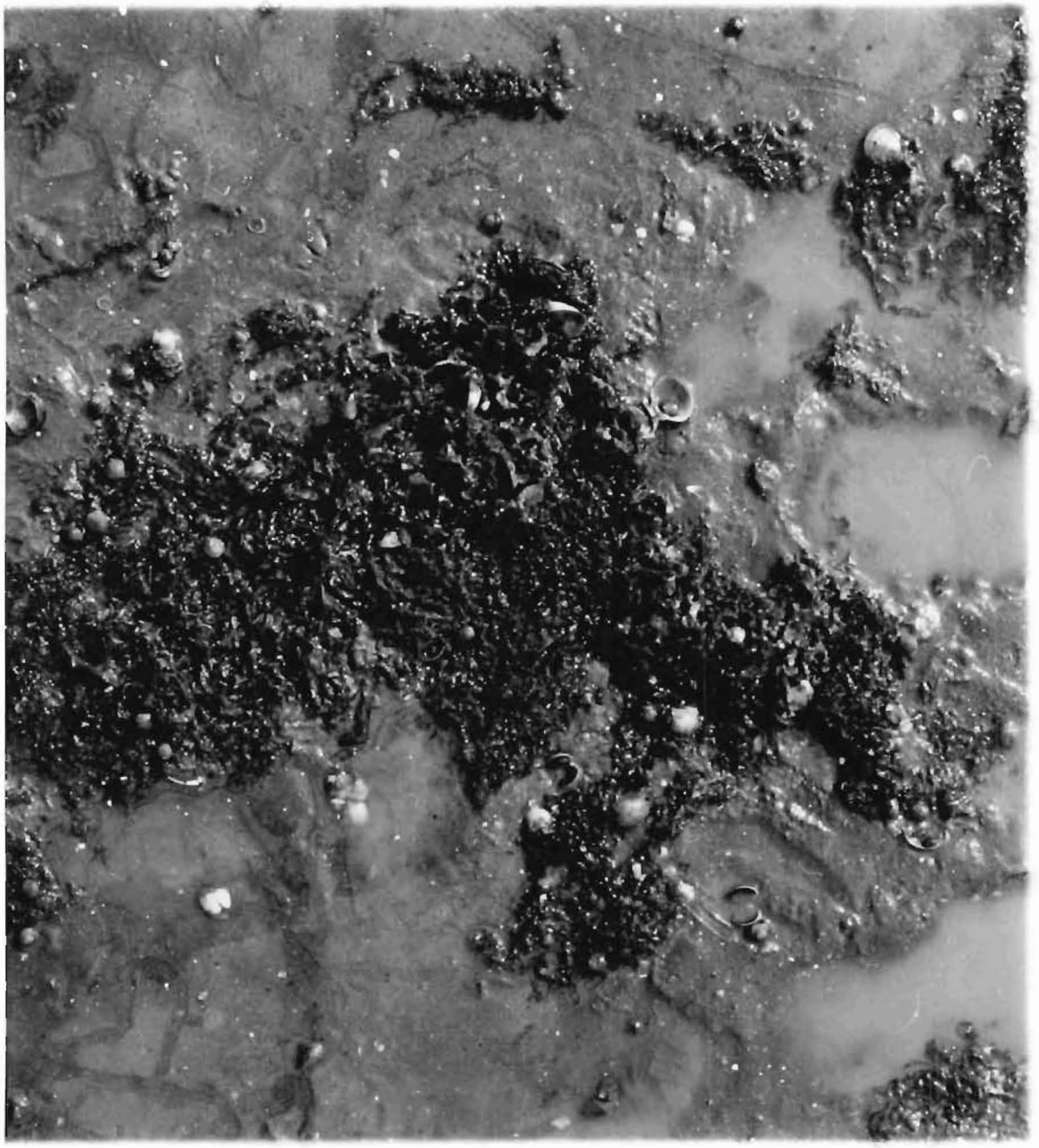


Plate 6.3

M^2 quadrat from Monck's Bay showing *Ulva lactuca* and *Gracilaria secundata*, May 1971.

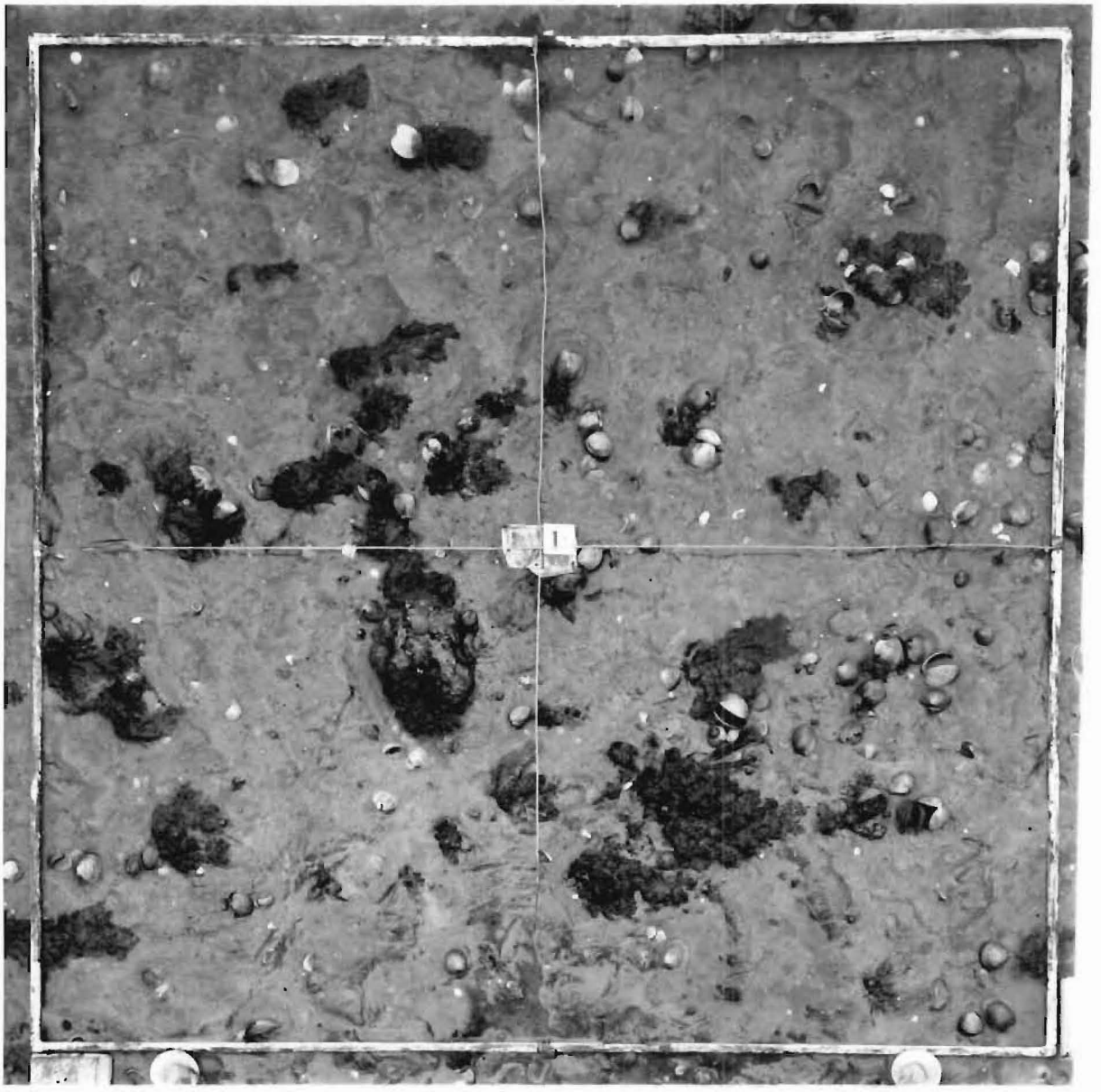


Plate 6.4

M^2 quadrat showing bullate Ulva lactuca at area C, May 1971. Note the small thallus size.

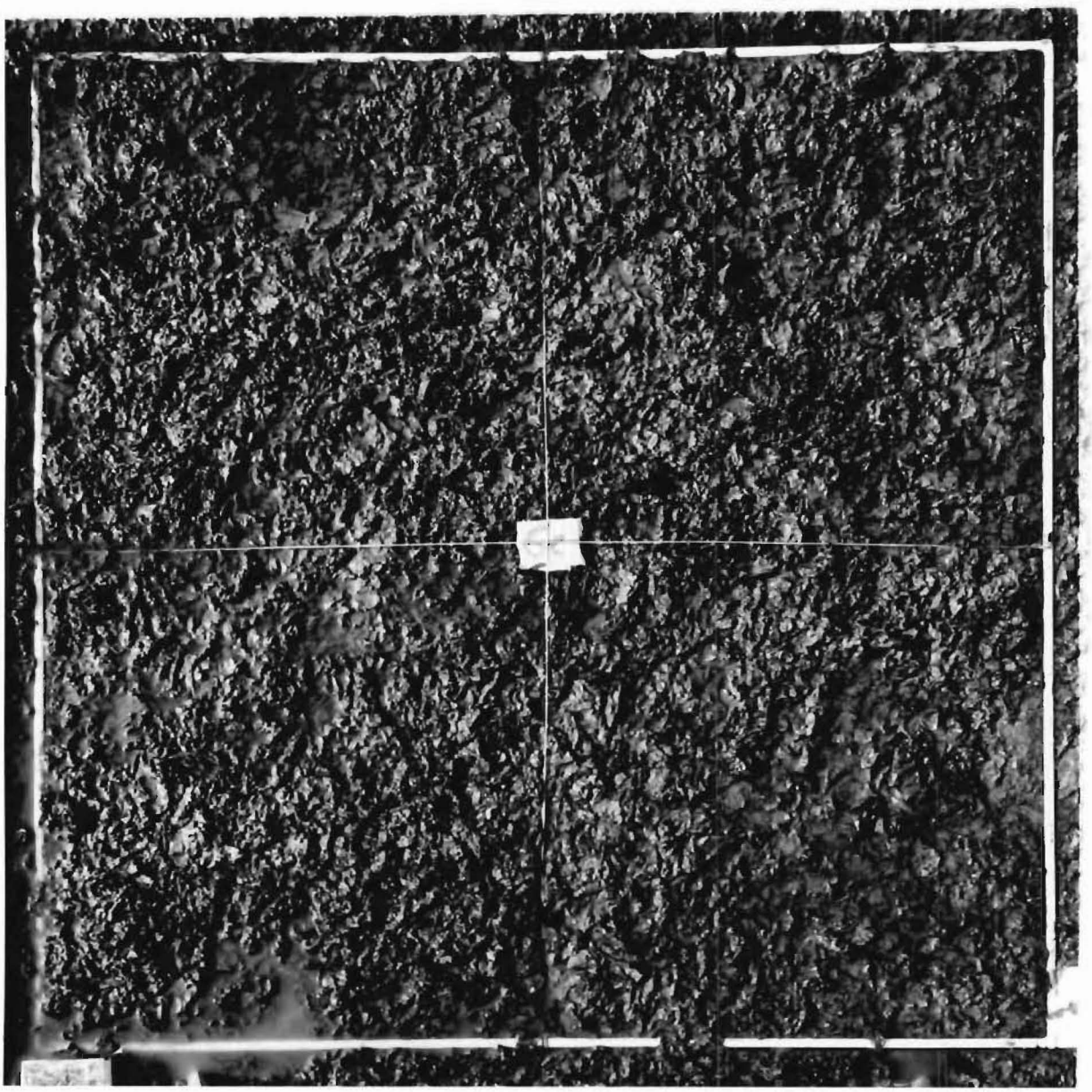


Plate 6.5

Same quadrat as in Plate 6.4 in March 1973.

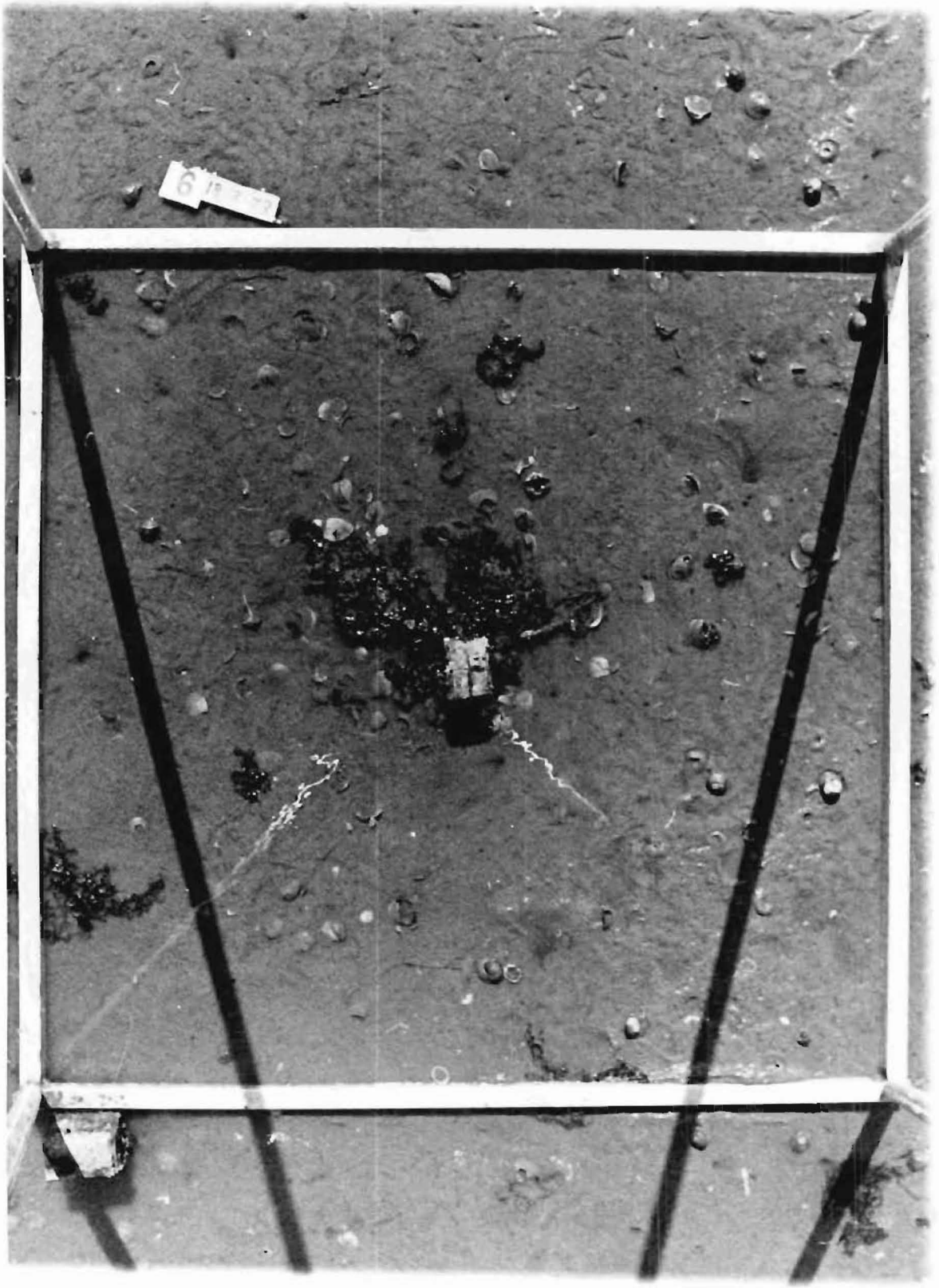


Plate 6.6

M^2 quadrat showing drift Ulva lactuca at area D, March 1972. Note the small thallus size.

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30/5/72

Plate 6.7

M^2 quadrat of drift Ulva lactuca at area F, November 1972. Note large size of thalli.

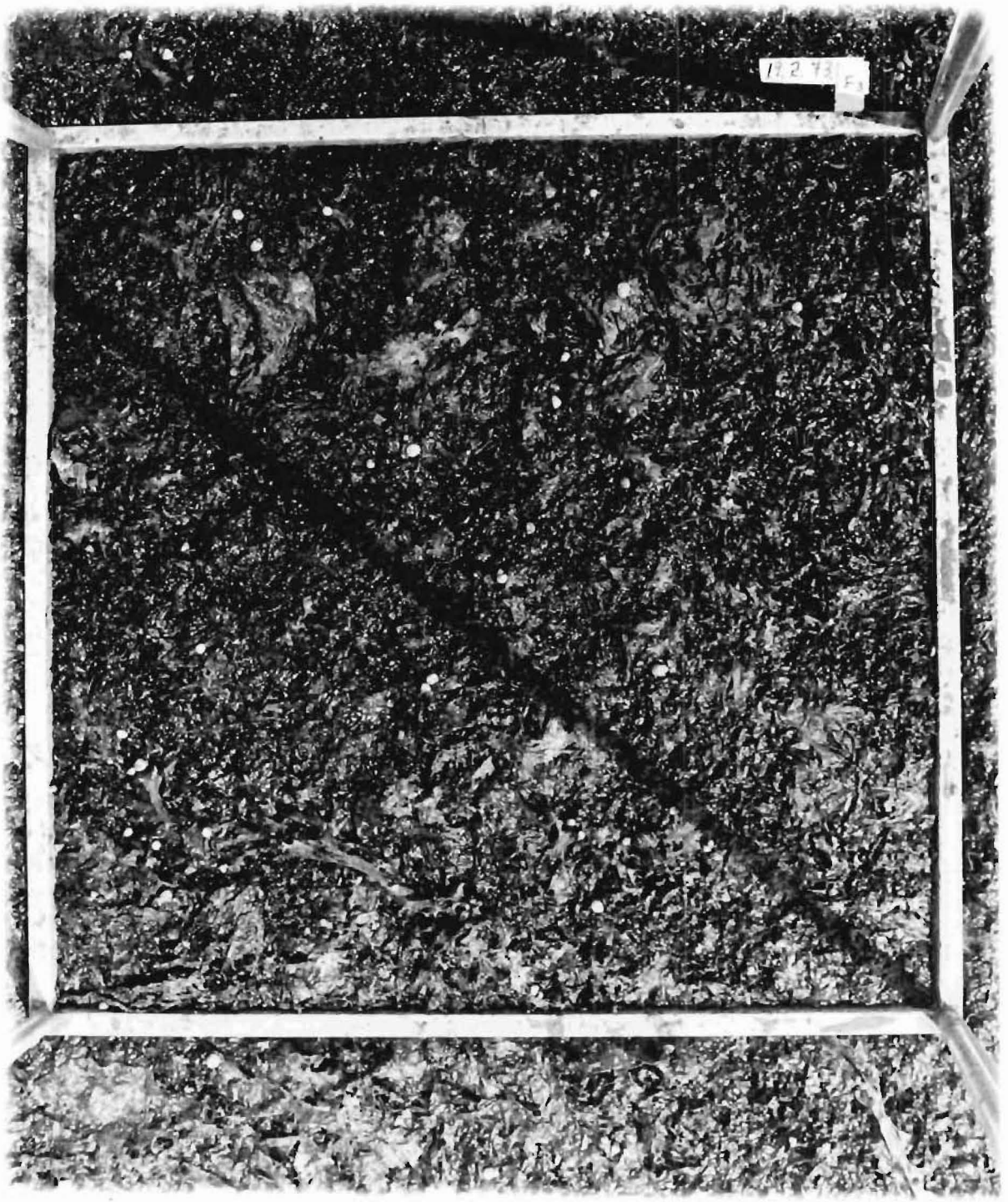


Plate 6.8

M^2 quadrat at area E showing Ulva lactuca and Gracilaria secundata, March 1973.

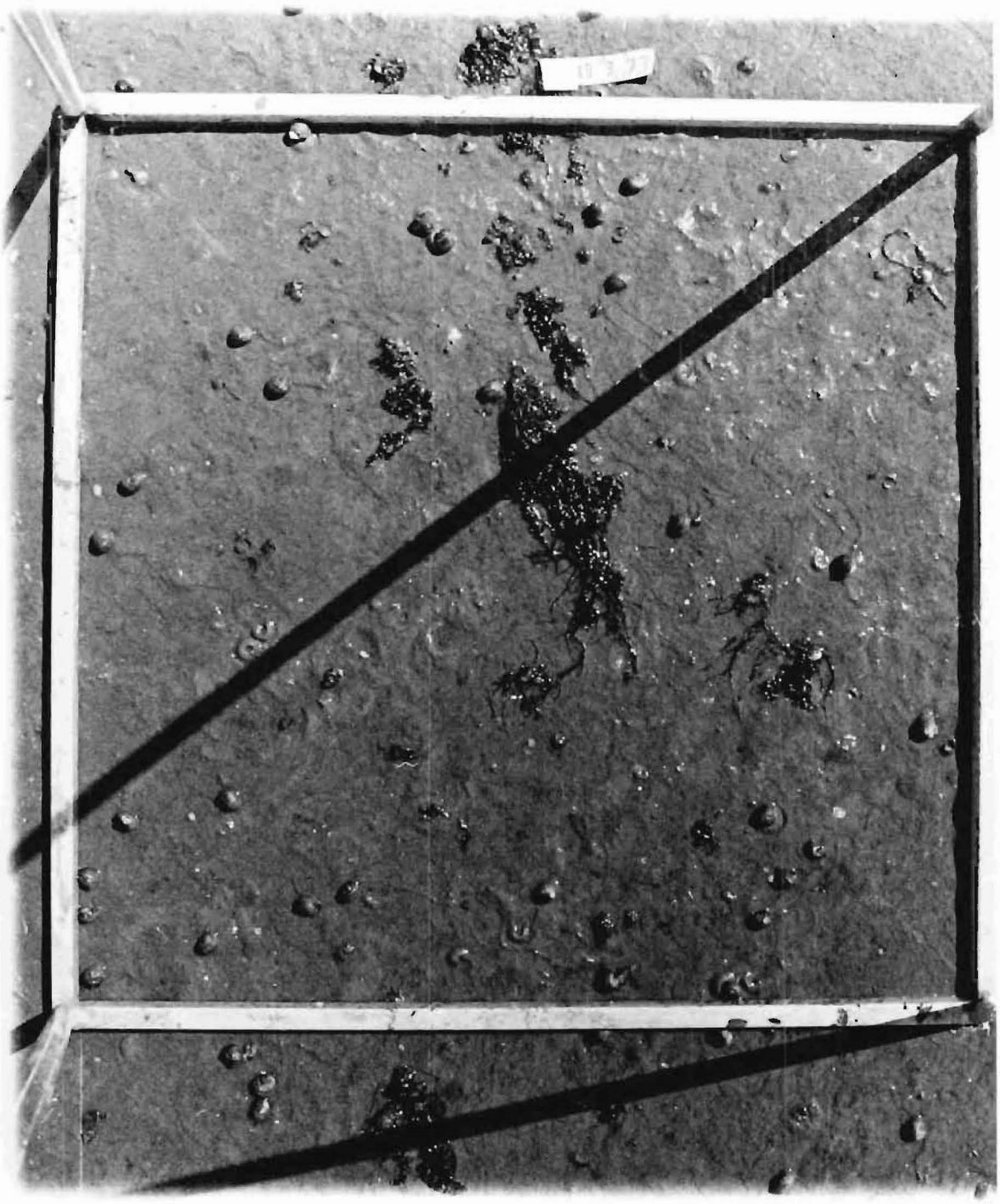
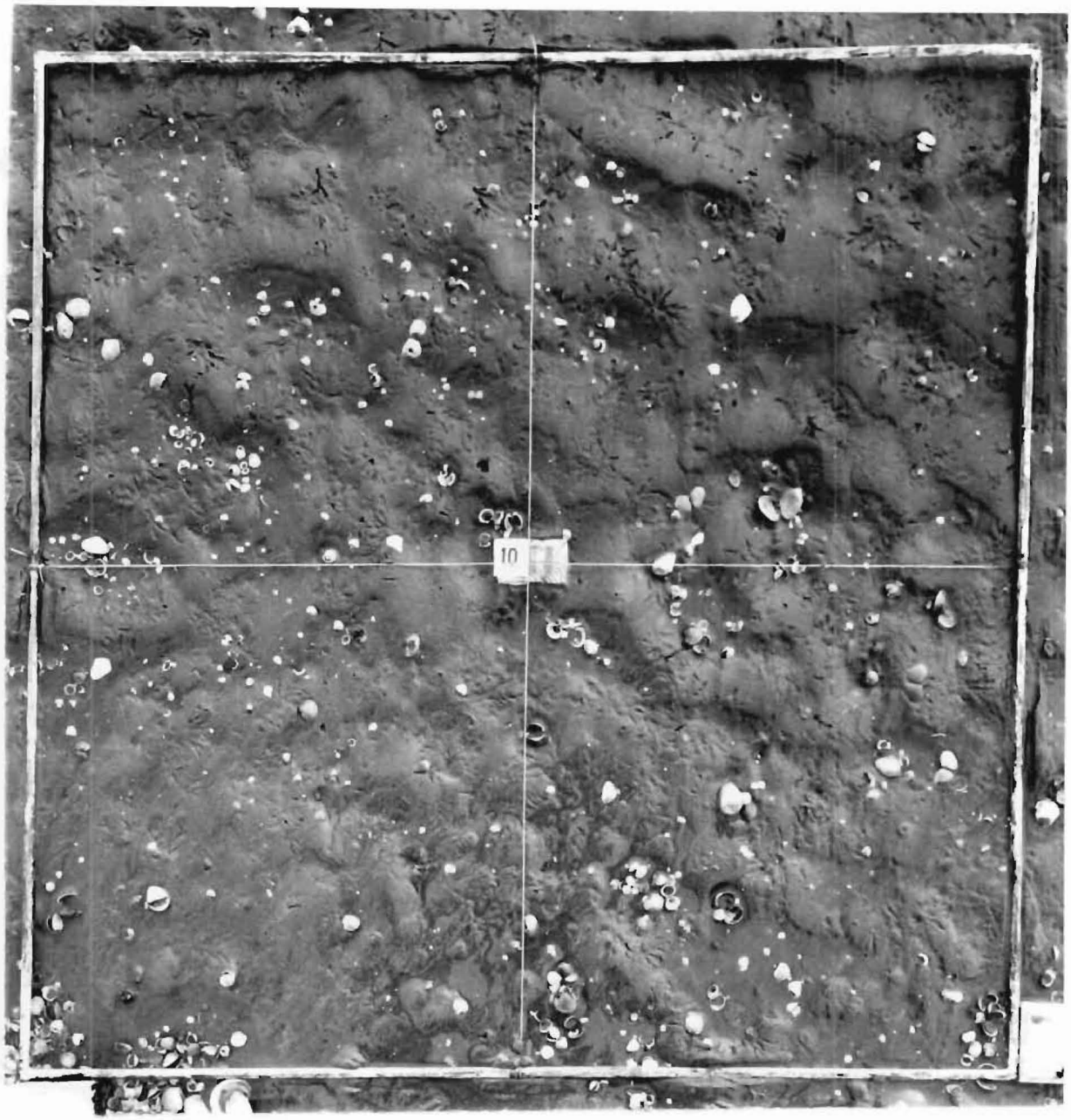


Plate 6.9

Quadrat 10, January 1972 showing a lack of algae despite the presence of shells.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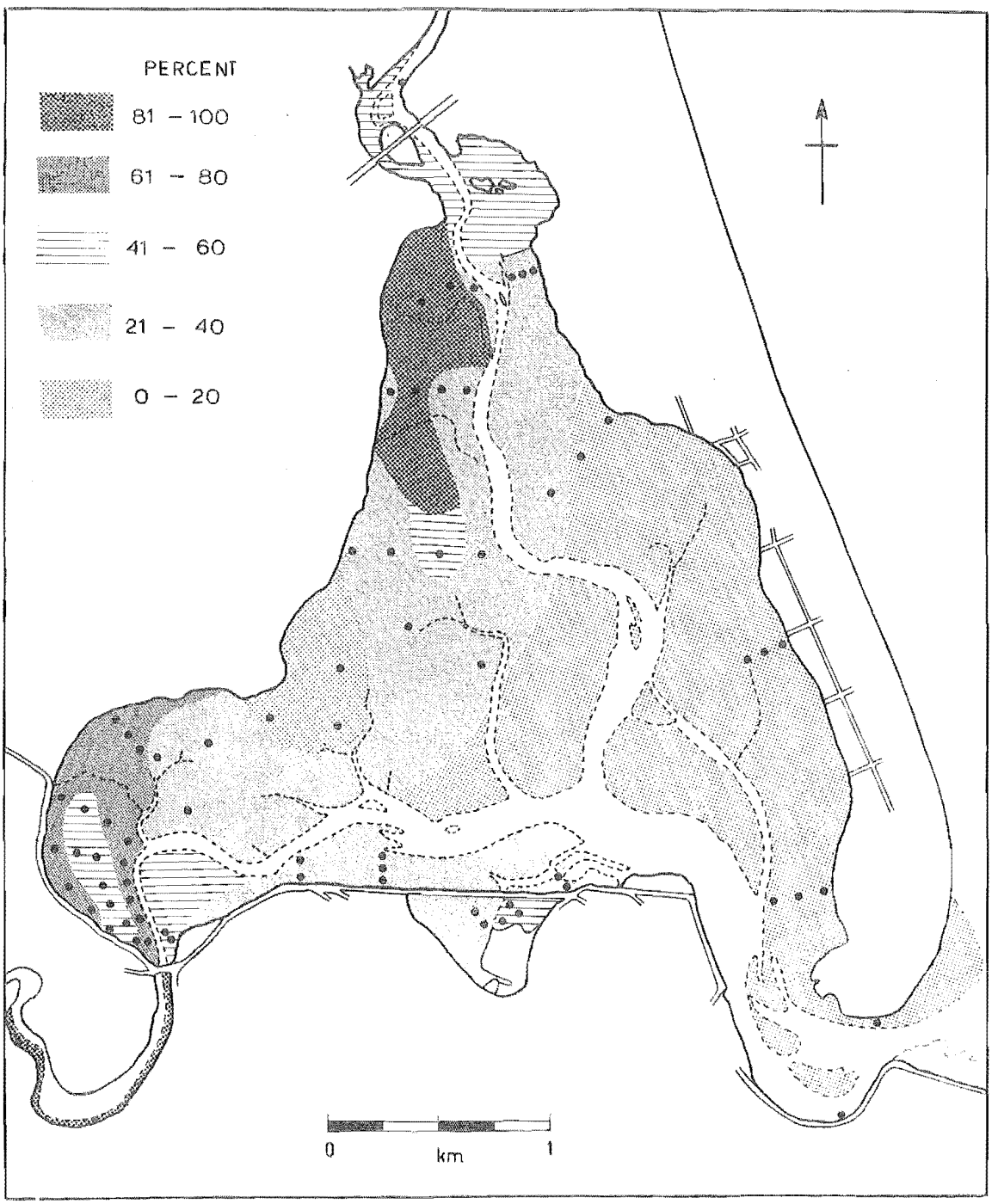


FIGURE 6.15 – SEDIMENT DISTRIBUTION IN THE ESTUARY SHOWING THE PERCENTAGE OF THE SILT CLAY FRACTION.

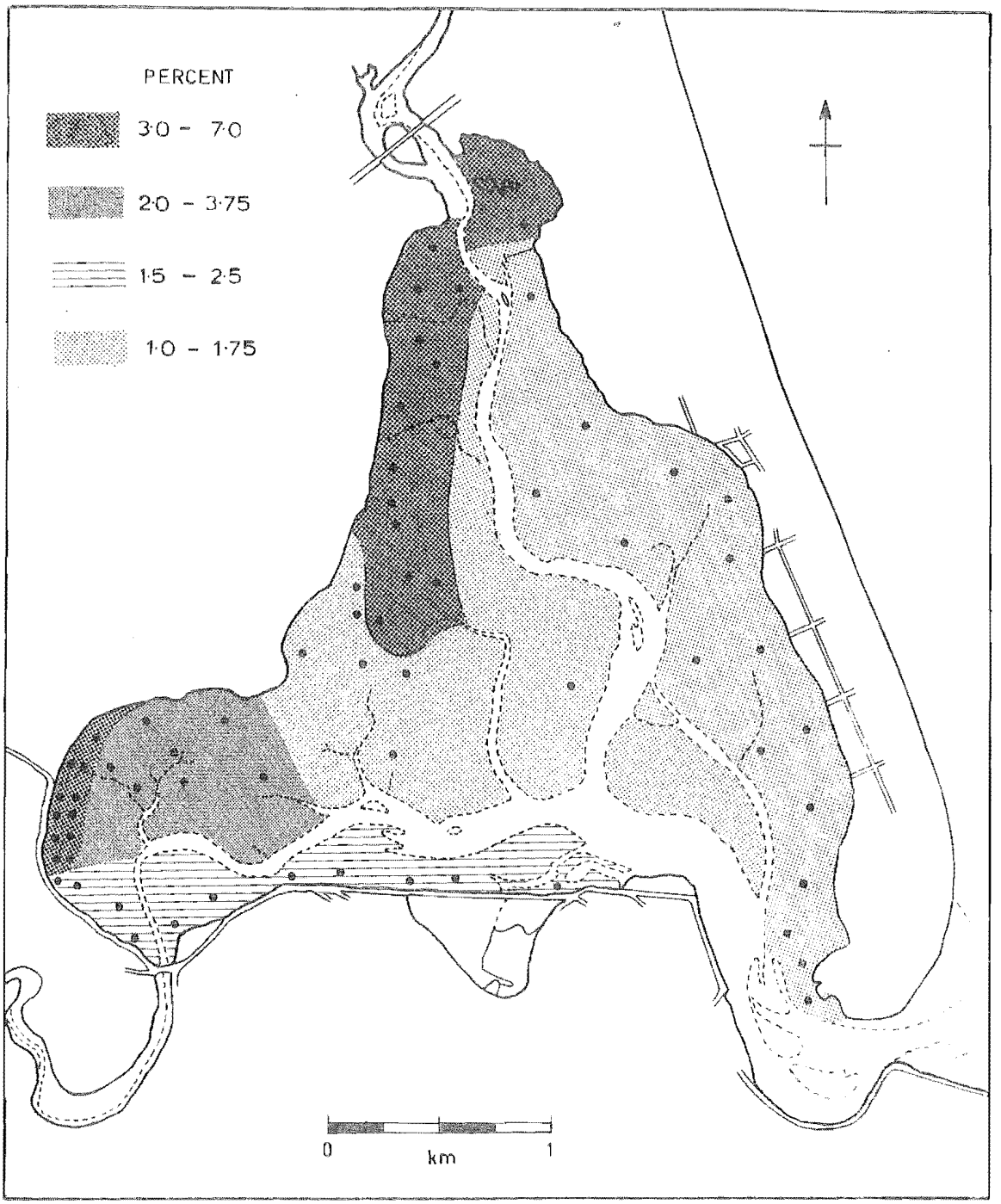


FIGURE 6.16 – PERCENT ORGANIC MATTER (AS A PERCENTAGE OF THE DRY WEIGHT) IN THE ESTUARY SEDIMENTS

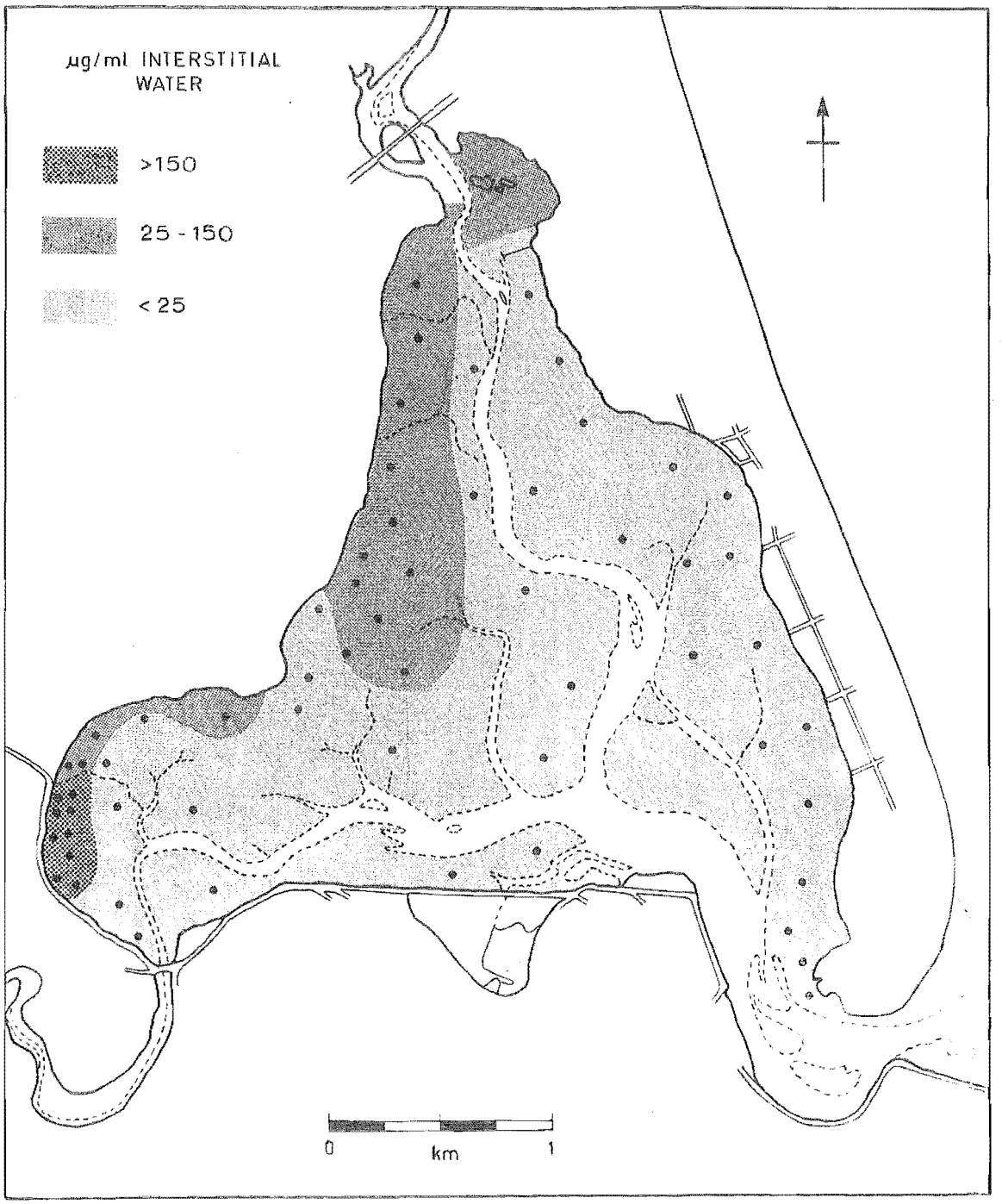


FIGURE 6.17 – AMMONIACAL NITROGEN DISTRIBUTION IN THE ESTUARY SEDIMENTS.

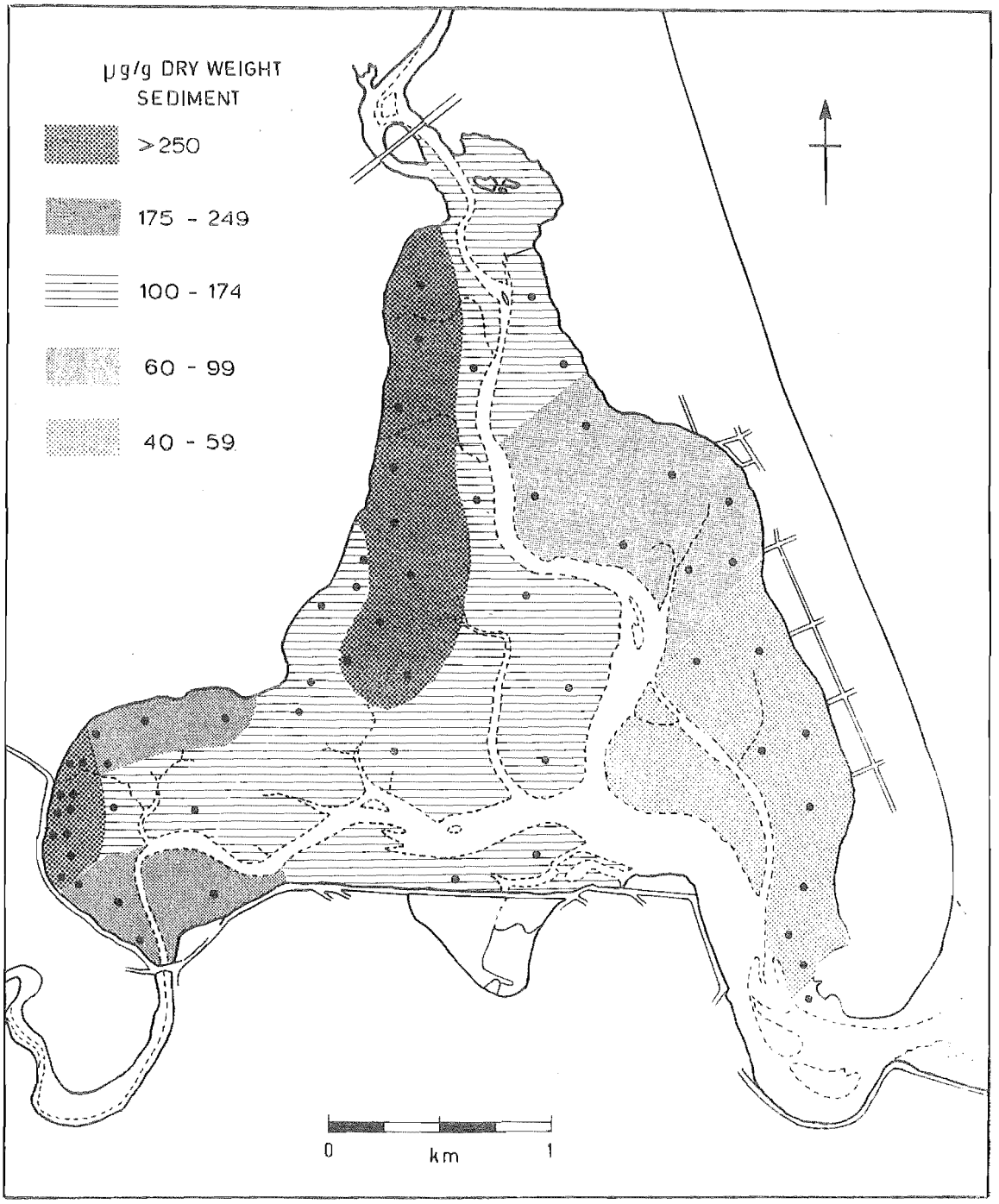


FIGURE 6.18 - ALBUMINOID NITROGEN DISTRIBUTION IN THE ESTUARY SEDIMENTS.

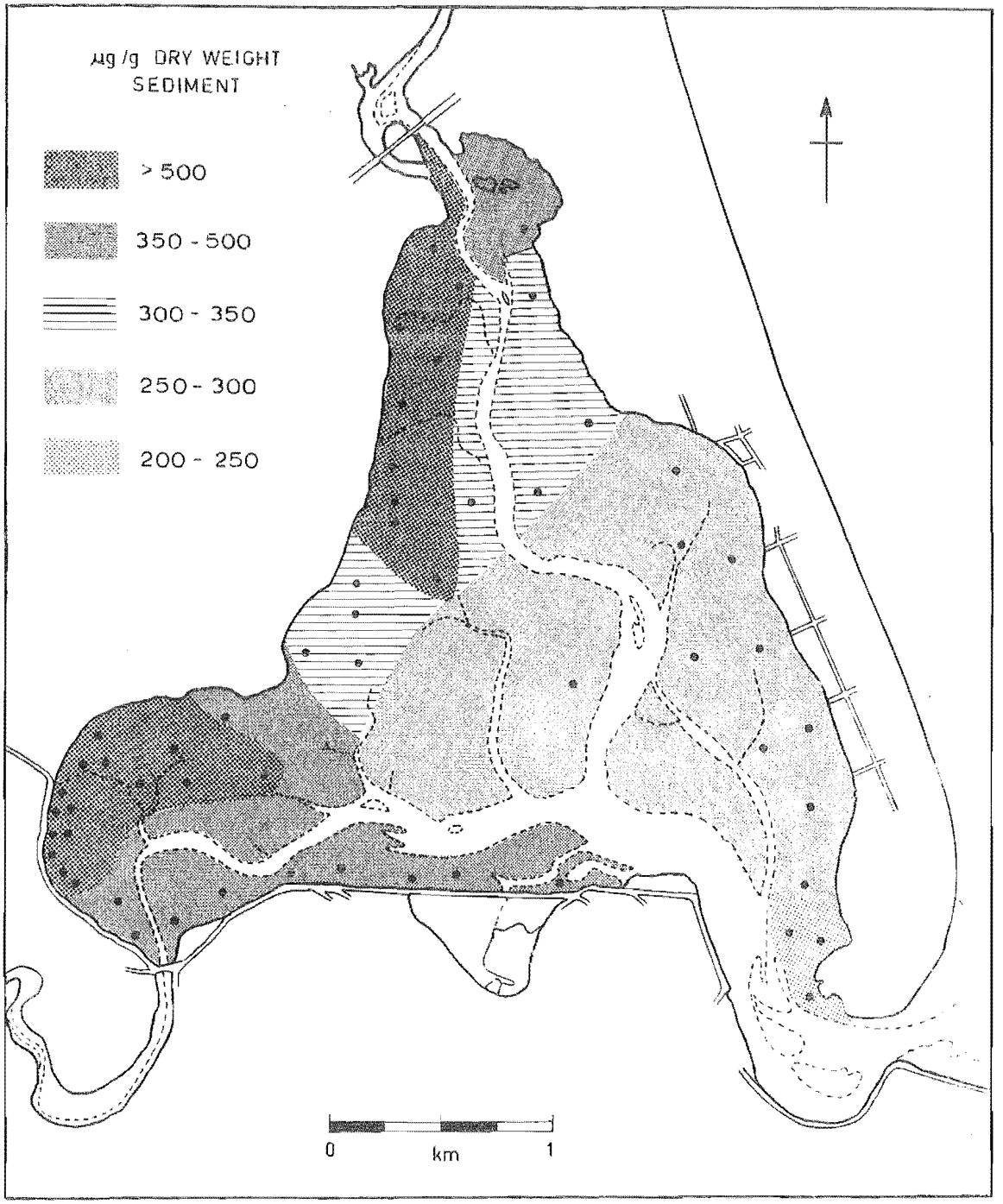


FIGURE 6.19 -TOTAL PHOSPHORUS DISTRIBUTION IN THE ESTUARY SEDIMENTS.

2) ENVIRONMENTAL FACTORS

(a) Substrate

The main attachment substrates for the algae are shell beds and assorted debris. The main concentrations of shells occur in beds at areas A, B and F and on Brighton Spit near the mouth of the Estuary. The remainder of the Estuary has a scattering of shells, but no dense beds. The sediment map of the Estuary was given by Knox and Kilner (1973) (Fig. 6.15). The percentage of the silt clay fraction increases near the outlet to the Bromley Oxidation Ponds and near the mouth of the Avon and Heathcote Rivers while the proportion of sand increases towards the mouth of the Estuary. The sands near the mouth of the Estuary are unstable and the pegs used to mark the sampling sites in this region were covered over after two to three months.

As shown in Figs 6.16 to 6.19 the percentage in the sediments of organic matter, albuminoid nitrogen and phosphorus, and the concentration of interstitial ammoniacal nitrogen all follow the same distribution as the silt clay fraction. The levels are very high in the fine sediments in the western region but decrease in the sandy substrates towards the mouth of the Estuary. Robb (1974) found that a linear regression of total phosphorus on organic content of the sediments gave a coefficient of 0 0153 x + 0.084 indicating a positive relationship between the two.

(b) Exposure/submergence pattern

The overall pattern of exposure derived from a series of aerial photomosaics is given in Fig. 6.20 (see Plates 6.10-6.13 for examples of the photomosaics). The greater part of the

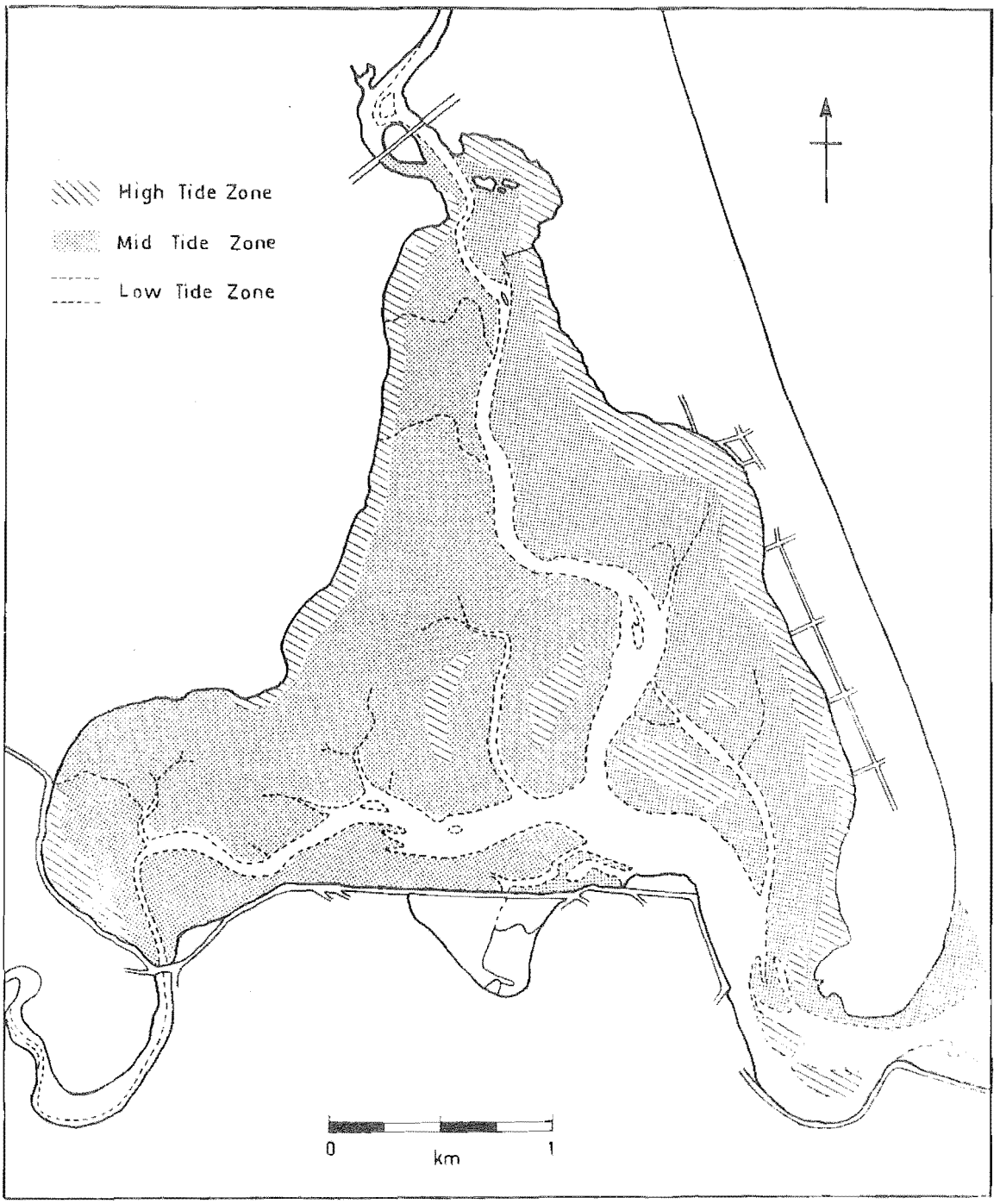


Fig. 6.20 Intertidal zones of exposure times from aerial photomosaics.
High tide zone > 8 hours exposure/tidal cycle.
Mid tide zone 4-8 hours exposure/tidal cycle.
Low tide zone (including channels) < 4 hours exposure/tidal cycle.

Estuary falls into the mid-tidal category with 4-8 hours exposure per tidal cycle. The main high tide zones occur on Brighton Spit, the central high areas and on the western shore. The exposure times for each sample area are summarised in Table 6.5.

Table 6.5 Exposure time at algal sample areas

		Sample area					
		A	B	C	D	E	F
Hours of exposure per tidal cycle	Range	4.5-5.5	5-6	6-7	6-7	8-10	5-6
	Mean	5	5.5	6.4	6.5	9	5.5

(c) Current patterns on the Estuary

The currents on the Estuary fall into two categories: those related to flow from the rivers and from the oxidation ponds, and those related to the ebb and flow of the tides. Webb (1972) investigated the direction of water movement at various states of the ebb and flood tides by observing the movement of plastic trawl floats 25 centimetres in diameter (Fig. 6.21). At the beginning of the flood tide the salt water follows the main channels before spilling over into the tributory channels at mid-tide. At high tide the main currents radiate out from the point where the channel to Shag Rock opens into the main body of the Estuary opposite Beachville Road. The currents parallel the shore line on Brighton Spit, but are split by the high areas in the centre of the Estuary. As a result, the currents on the western shore parallel the shore north of Sandy Point, but run south from Sandy Point into the bay north of the Heathcote River

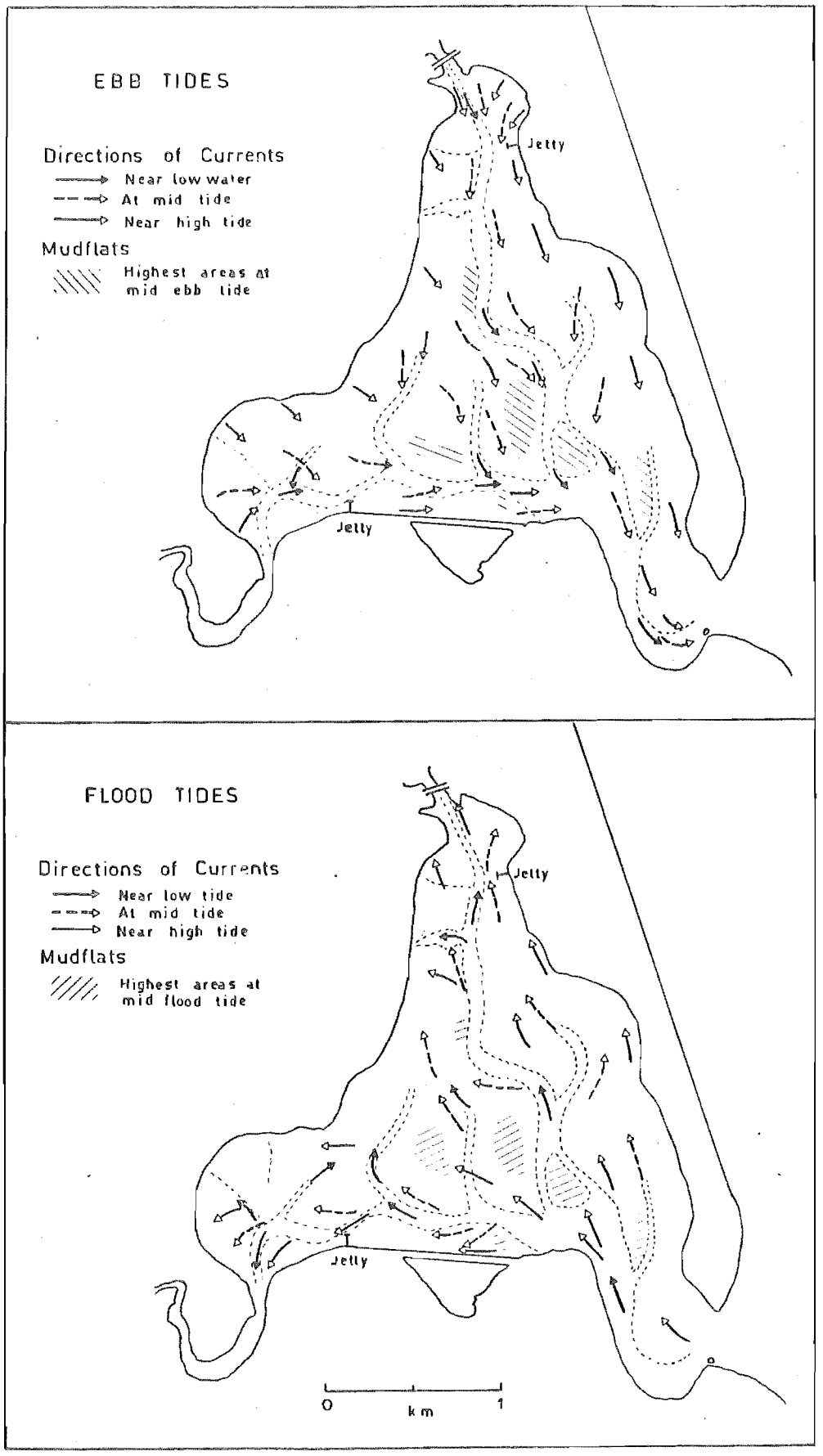


Fig. 6.21 Direction of water flow at various states of the tide in the Estuary (after Webb, 1972).

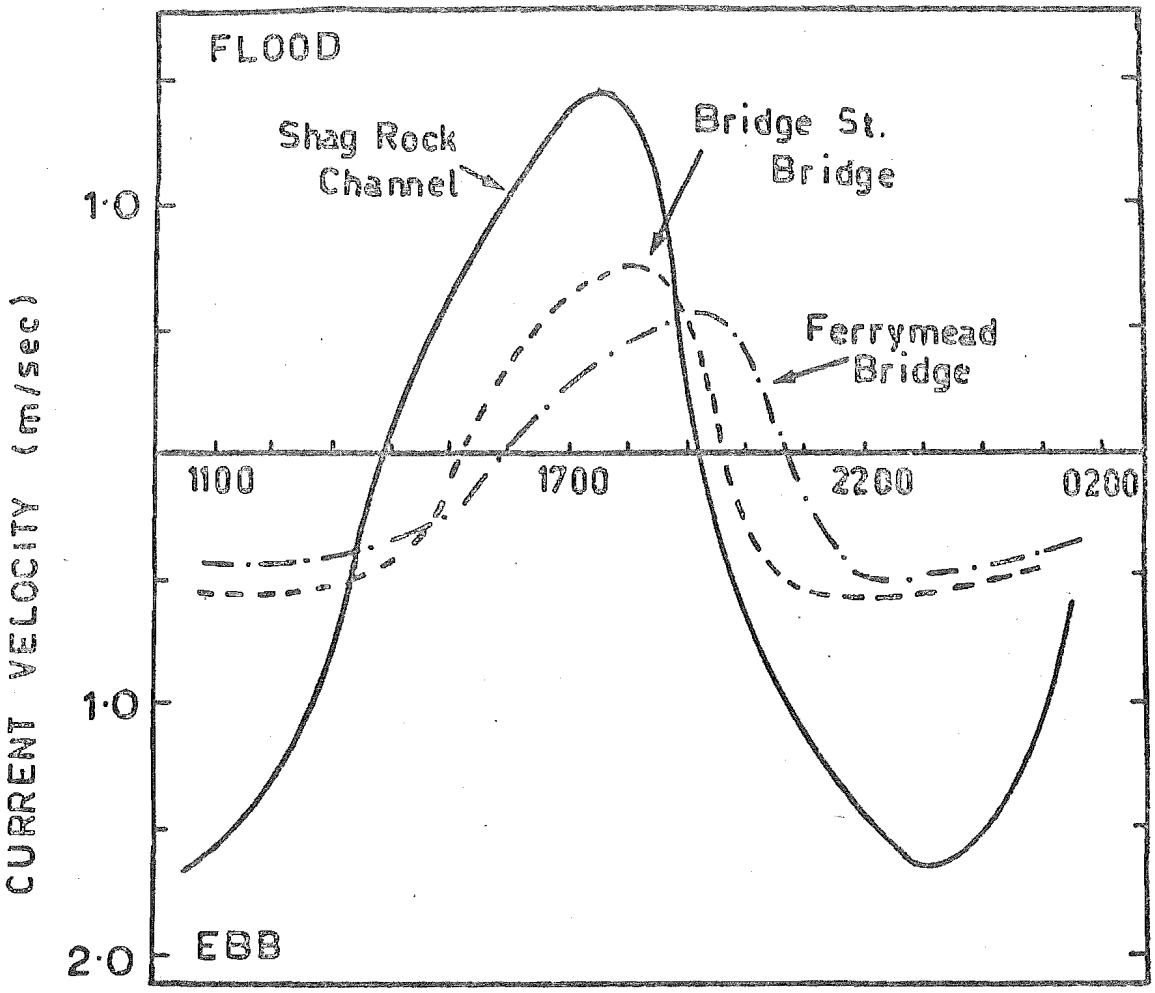


Fig. 6.22: Smoothed curves for mean current velocities over a tidal cycle at Shag Rock Channel, Ferrymead Bridge and Bridge Street Bridges (after Wallingford Report).

mouth.

The pattern on the ebb-tide is simpler, the water taking the shortest route to Shag Rock after high tide and following the channels at mid and low tide.

Current velocities: The velocity of the tidal currents varies both with location and the state of the tide. Fig. 6.22 shows the mean current velocities at the channel at Shag Rock and at the Bridges where the two rivers enter the Estuary. The river velocities at this time were low,

82 cusecs for the Avon and 18 cusecs for the Heathcote (Knox and Kilner, 1973). The flood tide occupies a shorter part of the tidal period with increasing distance from Shag Rock which is reflected in the lower ebb-tide current velocities.

(d) Dispersal of sewage effluent

As shown on Plates 6.11, 6.12 and 6.13, the effluent from the Bromley Oxidation Ponds tends to move up the Avon River on the flood tide but moves south towards the Heathcote River on the ebb tide. The observations of the dispersal of the dyes as shown on Plates 6.14 and 6.15 indicate that the dye released into the sewage effluent at high tide moved southward and entered the Heathcote River channel opposite Mount Pleasant, while the dye released in the Avon channel immediately opposite the Oxidation Ponds followed the main Avon River channel. The leading edge of the dye patch in the effluent reached Shag Rock 320 minutes after its release while the dye from the Avon River channel took 260 minutes to reach this point (Fig. 6.23). By the time the leading edge of the dye patch had reached Shag Rock, the trailing edge was still opposite McCormack's Bay indicating considerable differences in the rates of dispersal within each patch. Some smaller patches were also trapped in the mud flat pools by the ebbing tide.

The ebb tide covers 6 hours of the 12 hour 30 minute tidal cycle, while the effluent took 5 hours 10 minutes to reach the sea. Consequently only the effluent discharged within 50 minutes of high tide will reach the sea, which is only 15% of the effluent discharged on the ebb tide. Bruce (1953) estimated that 40% of the water leaving the Estuary

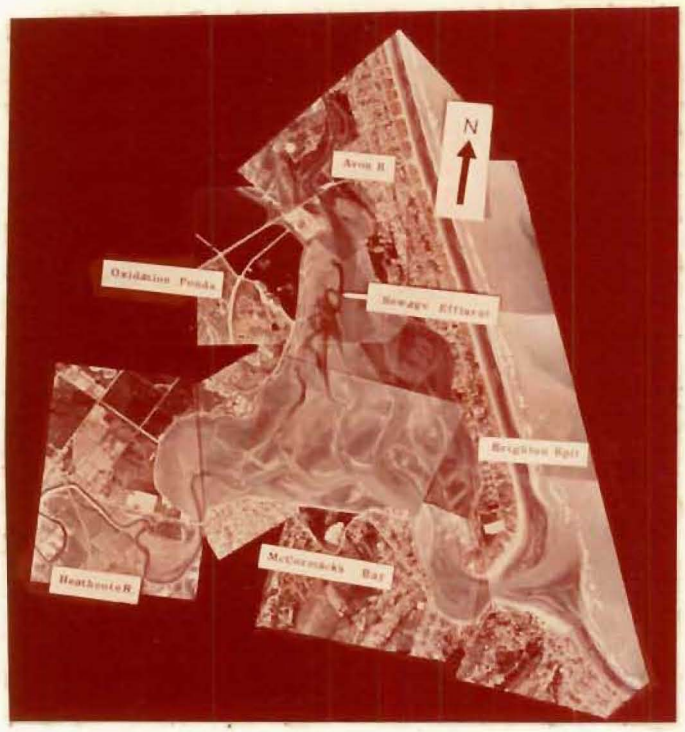


Plate 6.10 Aerial photomosaic showing sewage disposal at low tide.

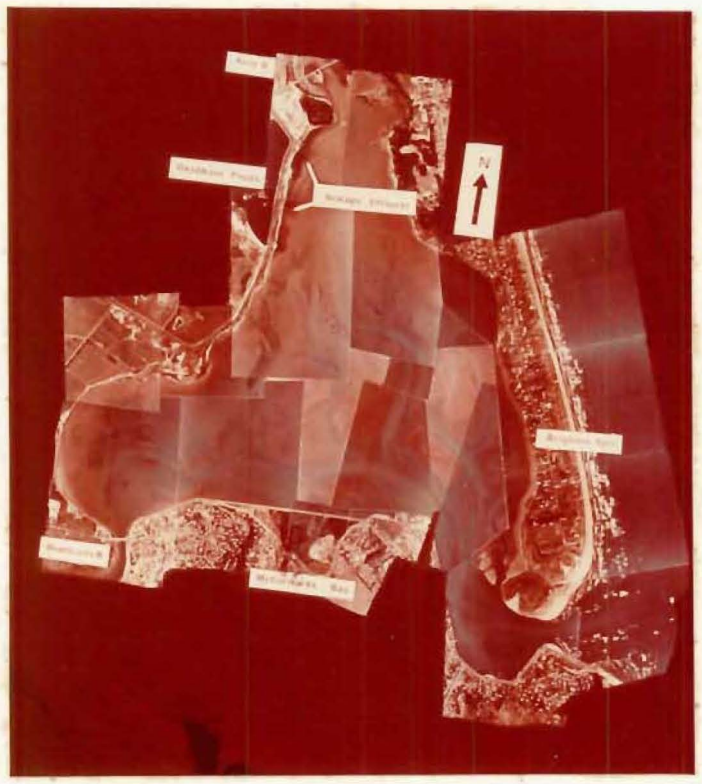


Plate 6.11 As for Plate 6.10 but on the flood tide.

Plate 6.12

Sewage dispersal
at high tide.

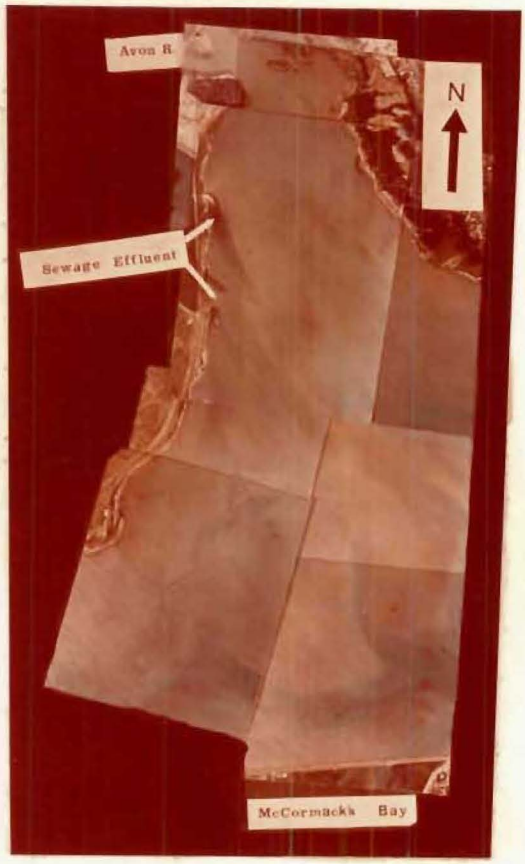


Plate 6.13

Sewage dispersal
on the ebb tide.

Plate 6.14 Oblique photograph of dye dispersal; fluoresciene in the fore-ground, rhodamine in the back-ground.

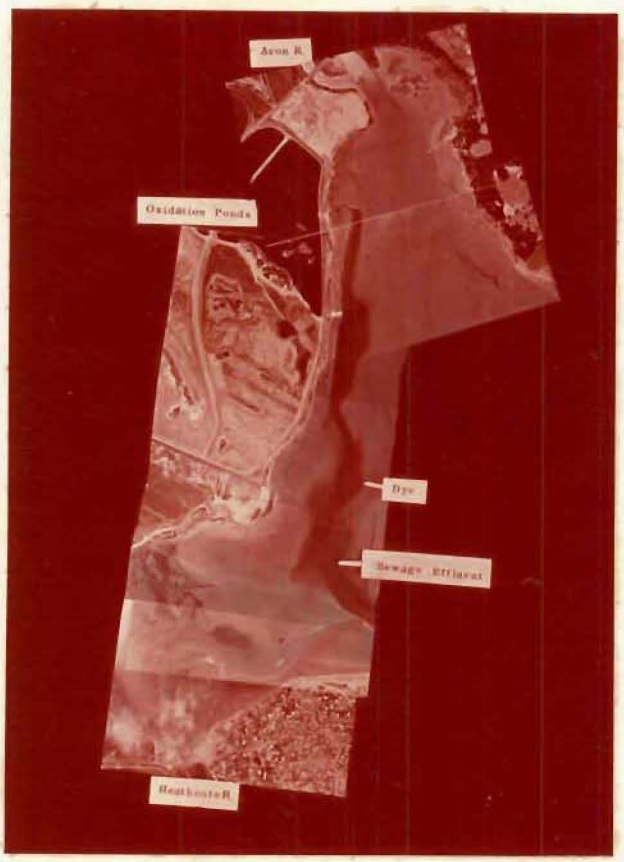


Plate 6.15 Vertical aerial photographic mosaic of rhodamine dye dispersal.

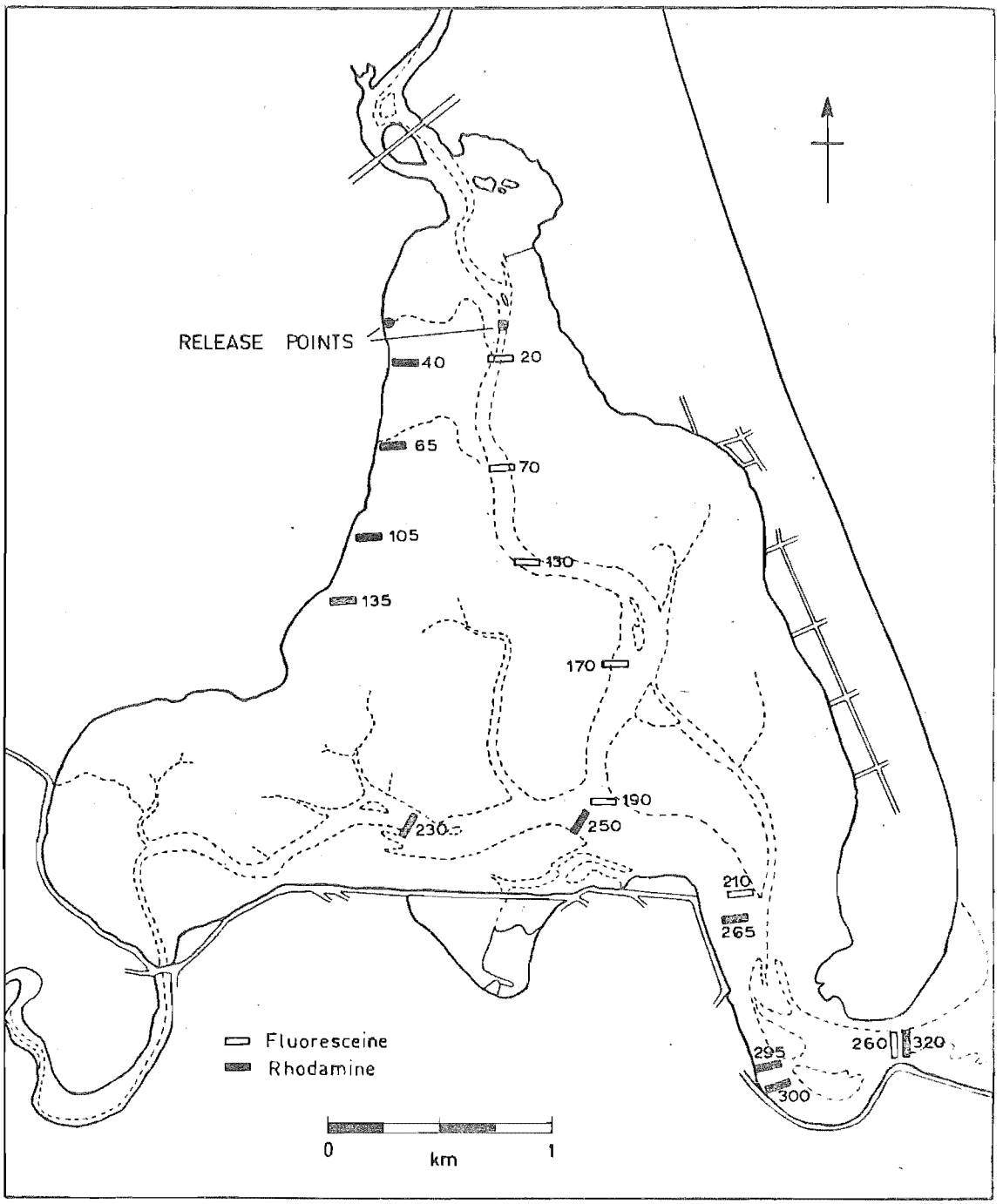


Fig. 6.23 Movement of the patches of rhodamine and fluoresceine dye. The positions of the leading edges of the dye patches are shown with the respective elapsed times in minutes after release.

returned on the following tide. As the returning water would consist of that which left the Estuary immediately before the tide turned, the amount of effluent leaving the Estuary without returning may be less than 15%.

(e) Sources of nutrient discharge into the Estuary

As summarised in Fig. 6.24, the major source of nutrients are the Bromley Oxidation Ponds, which supply 80% of the nitrogen and 90% of the phosphorus, the remainder coming from the rivers. The nitrogen contributions are shown in Table 6.6 from data collected by Wilkinson (1963) and by Robb in 1970-72 (Robb, 1974). The nitrogen is predominantly in the ammoniacal form due to the type of treatment at the Bromley Sewage Station. The increase in the proportion of ammoniacal nitrogen since the survey by Wilkinson in 1963 is probably due to the piping of sewage, previously discharged directly into the Estuary, into the main reticulation system (see Fig. 1.1 and Table 1.1).

The phosphorus is 80% reactive and 20% organic; again these proportions appear to be the result of transformations within the oxidation ponds.

Considerable quantities of microscopic freshwater algae are also released with the sewage effluent but as there is no information on their survival rate in salt water, one cannot assess their contribution to the nutrients.

(f) Nutrient levels in the water

Channel samples: (Table 6.8) The channel samples indicate that the nutrient levels increase towards the rivers and the oxidation pond outlets. This is the expected result as the eutrophic water from the rivers and the ponds would be

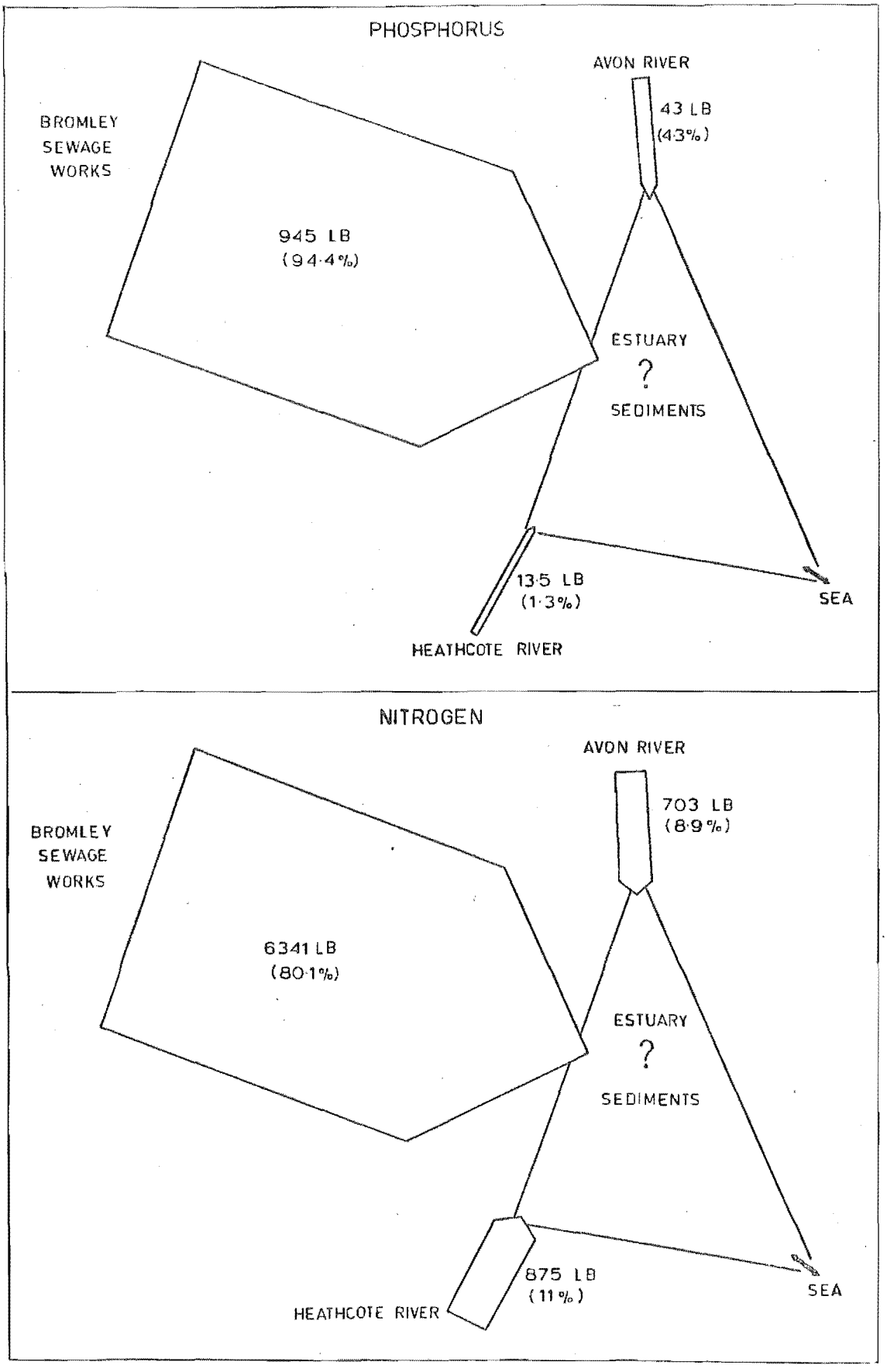


Fig. 6.24 The relative daily contributions of total phosphorus and nitrogen to the Estuary from the Bromley Sewage Works, the Avon River and the Heathcote River.

Table 6.6 Nitrogen contributions to the Avon-Heathcote Estuary: Wilkinson (1963) and Robb (1970-72).

(a) Nutrient loadings (kg/day)

	Nitrate	Nitrite	Ammoniacal Nitrogen	Organic Nitrogen	Total
(i) Wilkinson					
Heathcote	72.7	-	72.7	136.2	281.6
Avon	363.2	-	27.2	90.8	481.2
Sewage works	small	-	844.4	227	1071.4
Others	81.7	-	27.2	18.2	127.1
Total:	517.6	-	971.5	472.2	1961.3
(ii) Robb					
Heathcote	60.4	14.5	118.0	204.3*	397.2
Avon	121.7	3.2	43.1	151.2*	319.2
Sewage Treatment Plant	96.2	1.4	2176.0	605.2	2878.8
Total:	278.3	19.1	2337.1	960.7	3595.2

(b) Percentage composition

(i) Wilkinson					
Heathcote	3.7	-	3.7	7.0	14.4
Avon	18.5	-	1.4	4.6	24.5
Sewage works	small	-	43.0	11.6	54.6
Others	4.2	-	1.4	0.9	6.5
Total:	26.4	-	49.5	24.1	100.0
(ii) Robb					
Heathcote	1.7	0.4	3.3	5.7	11.1
Avon	3.4	0.1	1.2	4.2	8.9
Sewage Treatment Plant	2.7	small	60.5	16.8	80.0
Total:	7.8	0.5	65.0	26.7	100.0

* Albuminoid nitrogen x 3.

Table 6.7 Representative nutrient content (expressed as g/m^3) of river water at the points of entry to the Estuary (Robb, 1973).

	Heathcote	Avon
Ammoniacal nitrogen	1.4	0.12
Nitrate	0.80	0.5
Nitrite	0.15	0.01
Albuminoid nitrogen	0.8	0.2
Reactive phosphorus	0.04	0.05
Organic phosphorus	0.02	0.02

diluted by the relatively "clean" ocean water. The nutrient levels in the Estuary water could be expected to vary inversely with the proportion of ocean water in the sample. As the channel samples may have been taken at slightly different stages of the tide, they cannot be used as an indicator of seasonal change in nutrient levels.

Mudflat samples from above the algal sample areas: As shown on Fig. 6.25, the levels of nutrients vary considerably with the state of the tide. For each station the levels are highest just after the tide has covered the sampling site, decreasing sharply as the depth of water and salinity increase. With the exception of area C, the phosphorus and nitrate levels increase as the salinity decreases on the ebb-tide. From the graphs on Fig. 6.25, C tends to have the highest phosphorus and nitrate levels on the flood tide followed by D, B, E, F and A. At high tide all sites have similar nitrate levels, but some differ in phosphorus content. C tends to have higher phosphorus than B, D and E, which as a group are higher than A and F. On the ebb-tide the phosphorus levels at A, B, D and F increase, while that at C continues to drop.

Table 6.8: The range and mean concentrations of nutrients at the Estuary Channel Stations (in g/m^3). T = surface sample, B = bottom sample, N = number of samples, R = range, $\bar{x}$ = mean concentration.

		1T	2T	2B	3T	3B	4T	4B	5T	5B	7T	7B	9
Reactive P	N	29	22	21	20	18	43	26	21	21	43	24	29
	R	.002-.29	.002-.42	.002-.185	.002-.160	.002-.156	.002-.178	.002-.370	.002-.320	.002-.760	.002-.600	.002-.710	.002-.500
	$\bar{x}$	.079	.125	.098	.069	.059	.059	.062	.142	.157	.146	.119	.043
Total P	N	28	22	21	20	19	31	25	21	20	31	22	29
	R	.020-.800	.014-2.00	.010-.990	.016-.750	.013-.482	.030-.390	.032-1.25	.010-.688	.015-.454	.032-.77	.025-.63	.007-.49
	$\bar{x}$	.153	.268	.210	.182	.166	.149	.192	.278	.302	.255	.204	.086
NO_2^-	N	29	23	21	20	19	41	26	21	21	31	24	29
	R	.002-.12	.002-.04	.002-.03	.002-.06	.002-.05	.002-.093	.002-.07	.002-.09	.002-.1	.002-.08	.002-.053	.002-.011
	$\bar{x}$	.016	.013	.011	.018	.016	.036	.027	.012	.015	.019	.021	.004
NO_3^-	N	28	21	19	19	18	40	25	19	20	30	24	26
	R	.02-.25	.005-.30	.01-.170	.005-.52	.005-.52	.005-1.24	.005-.88	.01-.051	.02-.43	.005-1.20	.005-.60	.01-.70
	$\bar{x}$	.091	.100	.083	.162	.195	.432	.292	.204	.175	.314	.296	.074
NH_4-N	N	13	13	11	11	9	14	12	11	11	31	10	13
	R	.005-.28	.005-.52	.005-.54	.01-.83	.01-.52	.06-2.40	.08-1.54	.005-1.10	.005-1.20	.005-1.62	.005-1.14	.005-.27
	$\bar{x}$	.099	.214	.150	.286	.227	.729	.672	.630	.551	.530	.375	.068

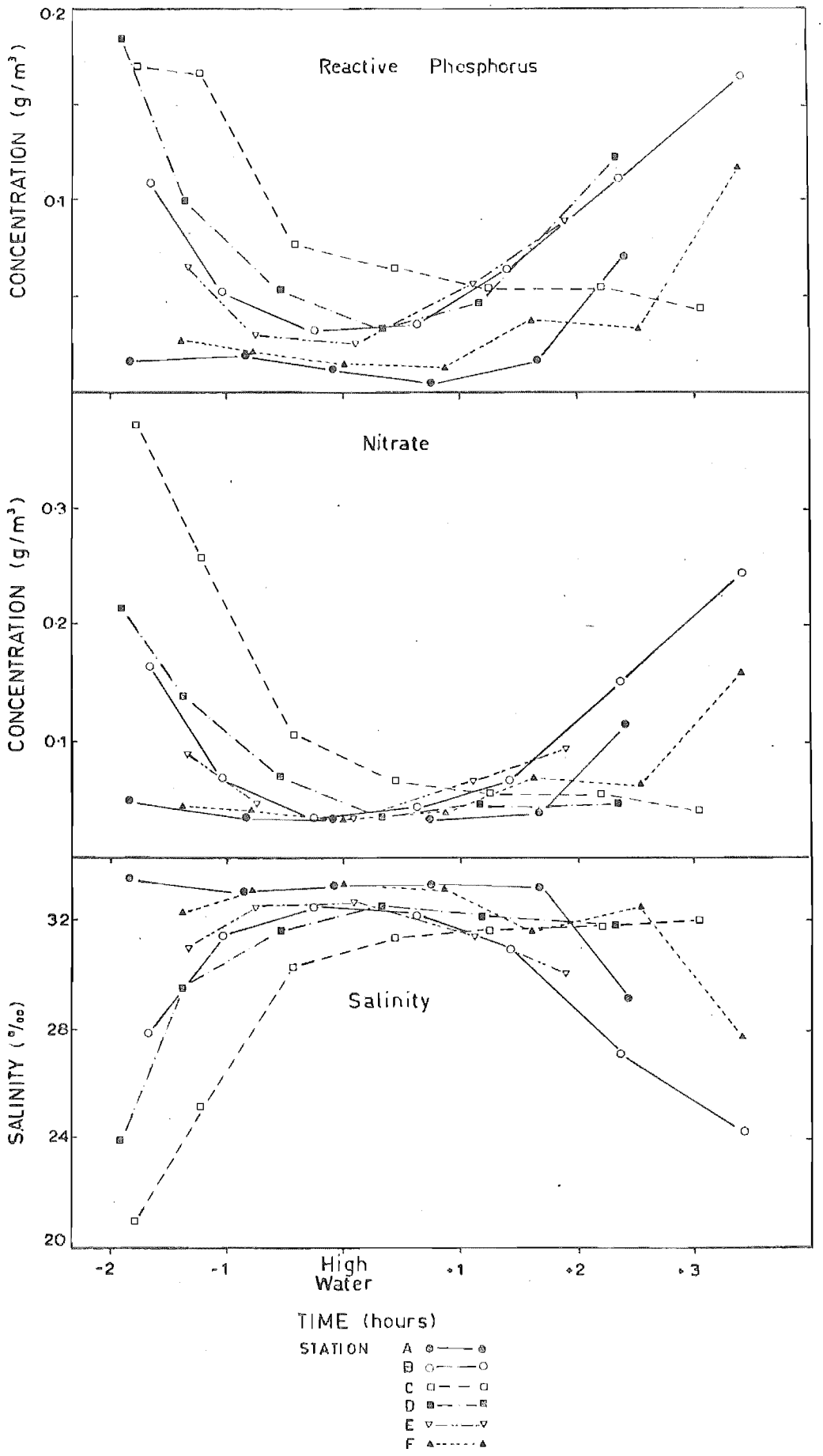


Fig. 6.25 Nutrient and salinity measurements over a full tidal cycle at the algal sample areas, December 1972.

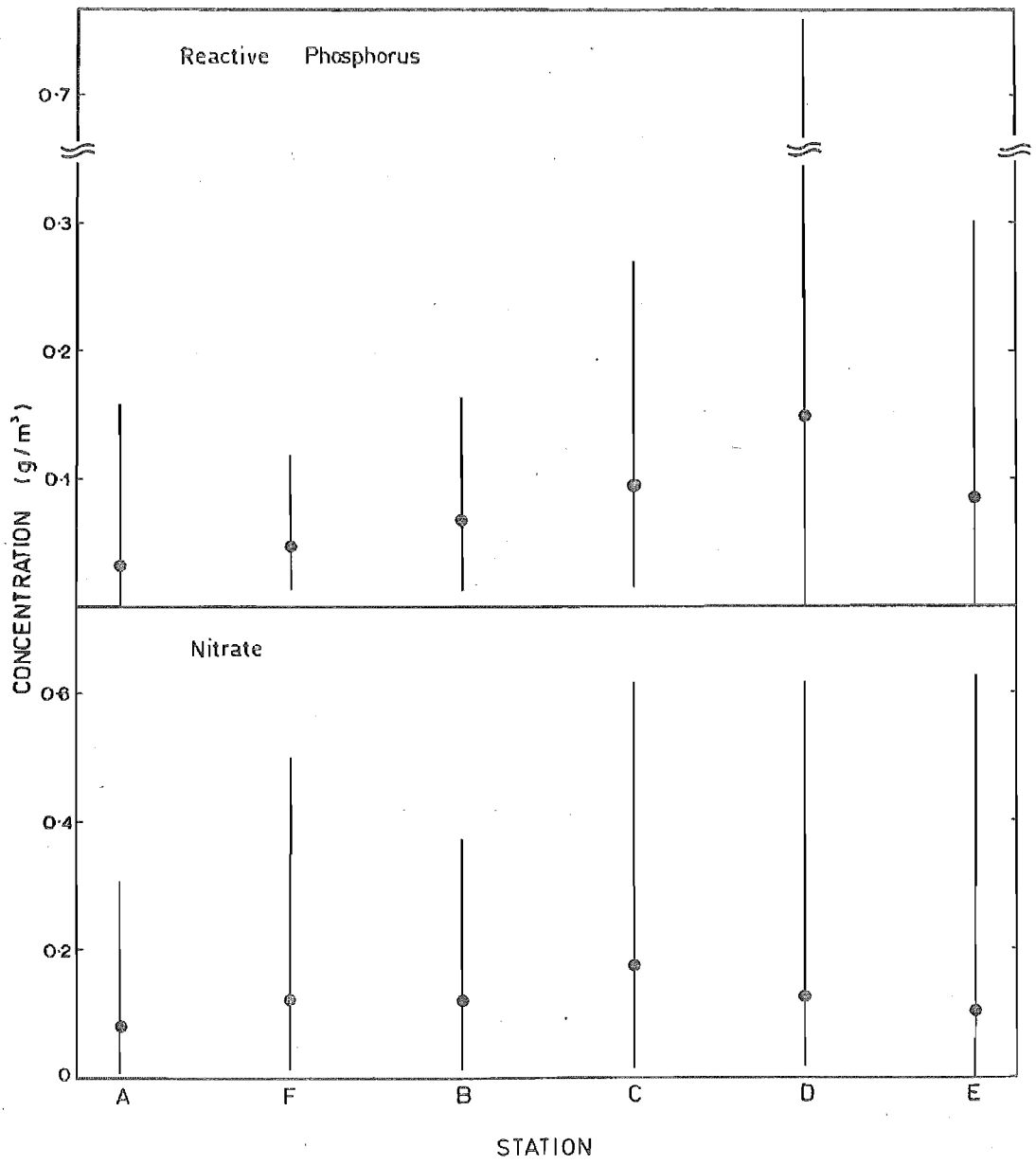


Fig. 6.26 The range and mean of reactive phosphorus and nitrate above the algal sample areas.

The nitrate level follows a similar pattern, except that concentrations at D and C are similar to each other. The salinity at C and D does not decrease on the ebb-tide as it does at A, B, E and F, possibly as a result of their greater distance from the rivers.

Seasonal variation in nutrient levels: The nutrient levels are inversely related to the proportion of ocean water to river water which will vary according to tidal heights with lower nutrient levels at spring tides than at neap tides. It is therefore necessary to compare data from tides of the same height when assessing seasonal variation. Table 6.9 gives the mean phosphorus levels from the three samples each for May, July and December 1972 around the high tide point when the tidal heights were all 1.8 and 1 ft at Lyttelton. In all cases the phosphorus levels are lower in July than in May and, with the exception of site A, the levels in May and December are similar.

Table 6.9 Seasonal distribution of phosphorus monthly means of the 3 samples closest to high tide in g/m^3.

	STATION				
	A	B	C	D	E
May 1972	.026	.038	.066	.039	.044
July 1972	.020	.028	.039	.028	.028
December 1972	.016	.040	.065	.044	.037

The range and mean nutrient levels at sites A to E for all months (November 1971 to January 1973) are shown on Fig. 6.26.

(g) Meteorological data

The mean monthly air and grass temperature from the Bromley Sewage Treatment Station and the water temperature from the Estuary are shown on Figs 6.27 and 6.28. The grass temperature is lower than the air temperature, which in turn was slightly lower than the water temperature on the Estuary. The maximum air temperature occurs in February: 12°C in 1971, 9.0°C in 1972 and 10.7°C in 1973. The minimum was -2.4°C in May 1971 and -2.1°C in June 1972. The mean for June, July and August 1972 was lower than for the corresponding period in 1971. The Estuary water temperature follows a similar pattern to that of the air temperature. These results agree with the pattern described for the Estuary by Estcourt (1962) and for Lyttelton Harbour (Skerman, 1958) (see Fig. 6.29).

The records from Bromley since 1967 are summarised in Table 6.10. The summer temperatures are higher than average in 1969/70 and 1970/71 and lower than average in 1971/72 and 1972/73. The autumn temperatures are less variable, only 1971 being noticeably lower than average. The winter temperatures were relatively high in 1971, while 1972 was colder. The spring temperatures were relatively high in 1969 and 1972, but low in 1968.

The mean monthly insolation in langleys and the hours of sunshine are given in Fig. 6.30. Although taken at Christchurch Airport, which is 9 miles from the Estuary, they should give an indication of the pattern of variation. The maxima for both insolation and hours of sunshine occur in January while the minimum values occur in June with a greater range in insolation than hours of sunshine. The decreases in

Table 6.10 Mean monthly temperatures at Bromley since 1967

(a) Grass temperature (°C)

	1967-68	1968-69	1969-70	1970-71	1971-72	1972-73	Grand mean
Dec.	9.7	9.9	11.2	9.6	10.3	7.2	
Jan.	10.6	10.7	12.0	12.6	9.0	10.5	
Feb.	10.4	10.1	9.9	12.0	9.2	10.7	
$\bar{x}$	10.33	10.3	11.0	11.4	9.5	9.5	10.3
March	-	7.7	10.0	9.2	9.3		
April	4.4	4.5	6.4	5.2	6.7		
May	4.1	3.6	3.2	-2.4	1.2		
$\bar{x}$		5.2	6.5	4.0	5.7		5.3
June	1.1	-2.4	1.1	3.0	-2.1		
July	-1.1	-1.1	0.2	1.6	0.7		
Aug.	0.5	0.3	1.3	2.0	-0.6		
$\bar{x}$	0.2	-1.1	0.81	2.2	-0.9		0.24
Sept.	0.5	5.0	2.3	1.8	2.2		
Oct.	2.5	4.3	4.4	3.9	4.0		
Nov.	4.65	8.1	6.3	7.1	8.4		
$\bar{x}$	2.6	5.6	4.3	4.3	4.9		4.3

(b) Air temperature (°C)

	1967-68	1968-69	1969-70	1970-71	1971-72	1972-73	Grand mean
Dec.	15.9	14.5	17.5	16.6	16.8	14.4	
Jan.	17.0	17.1	18.0	17.2	16.7	17.0	
Feb.	16.0	16.5	16.9	17.9	15.4	18.0	
$\bar{x}$	16.3	16.0	17.5	17.2	16.3	16.5	16.6
March		16.0	15.9	15.7	16.9		
April	11.9	12.1	13.8	12.0	13.8		
May	10.9	10.0	8.8	11.2	8.6		
$\bar{x}$	-	12.7	12.6	13.0	13.1		12.8
June	7.4	5.8	7.8	8.3	5.8		
July	5.7	6.0	7.6	6.7	6.9		
Aug.	8.8	7.2	8.8	8.9	6.7		
$\bar{x}$	7.3	6.3	8.1	7.96	6.5		7.14
Sept.	9.3	11.95	10.8	10.3	11.5		
Oct.	12.1	11.0	12.5	12.8	12.4		
Nov.	14.15	14.4	14.6	14.0	15.5		
$\bar{x}$	11.86	12.1	12.6	12.4	13.1		12.4

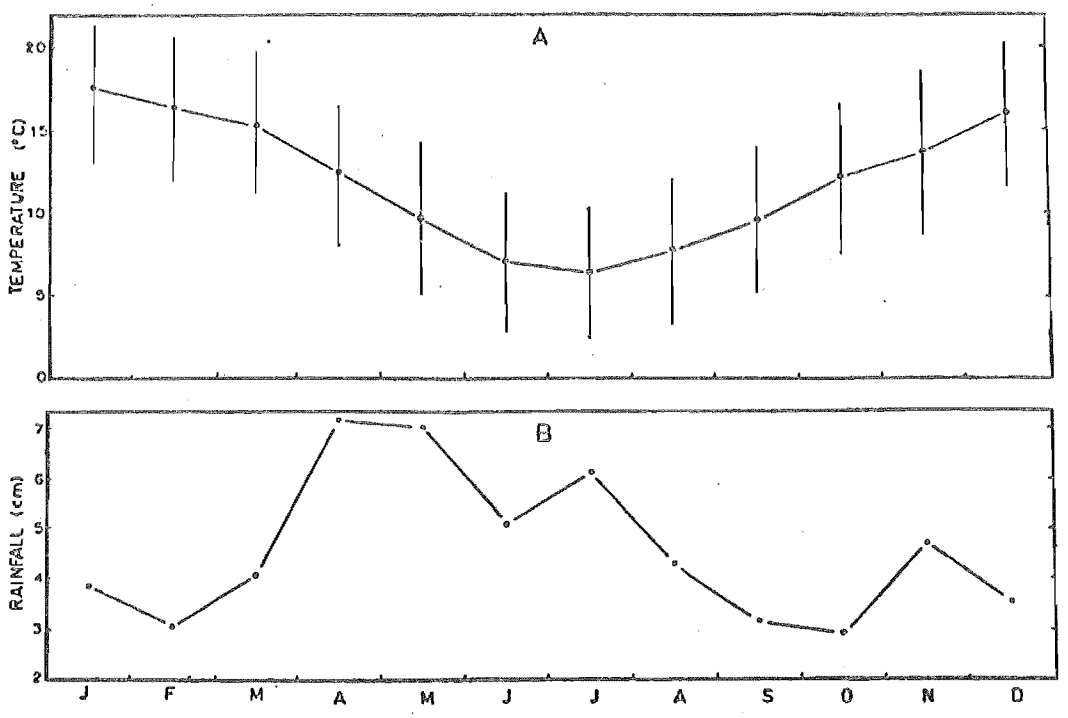


Fig. 6.27: A. Mean maximum, mean mean, and mean minimum monthly air temperatures at Bromley Sewage Works over a ten year period, 1962-1972.

B. Mean monthly rainfall recorded at Bromley over the same period.

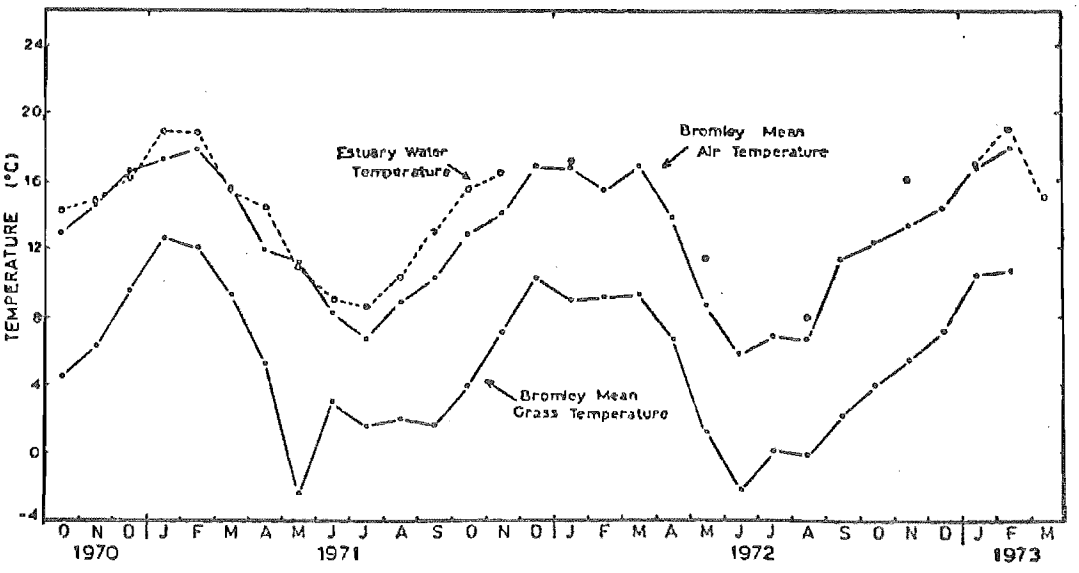


Fig. 6.28: Mean air and grass temperatures recorded at Bromley Sewage Works compared with water temperatures in the Estuary.

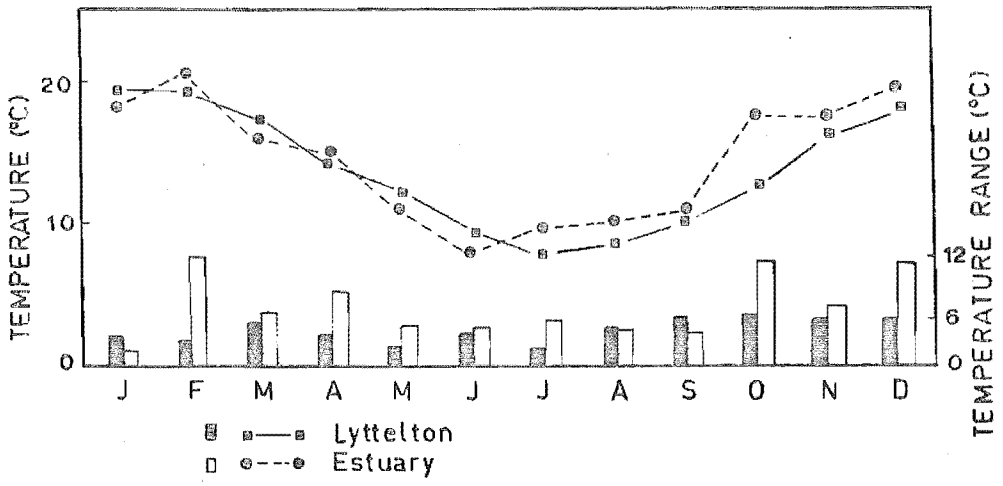


Fig. 6.29 Sea surface temperatures for Lyttelton (data from Skerman, 1958) and water temperature in the Estuary recorded in 1971 (Escourt, 1962).

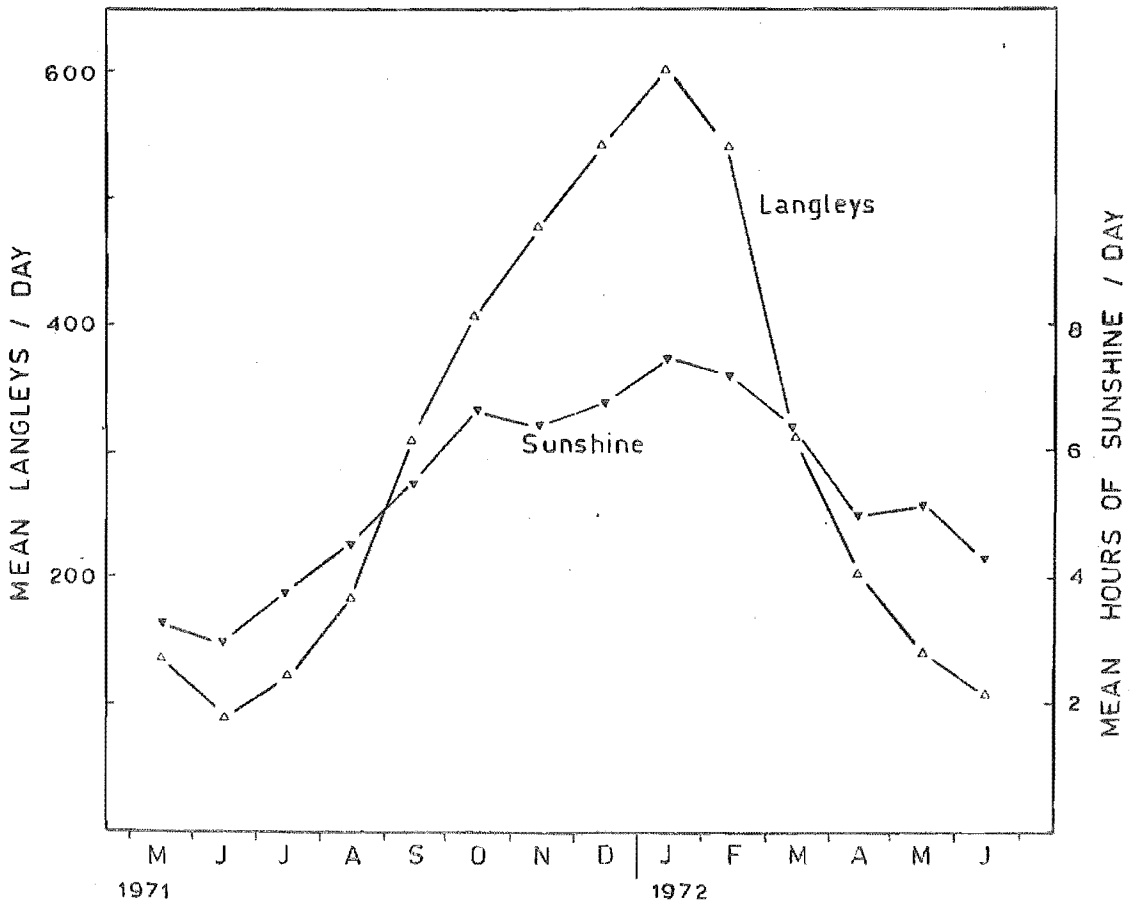


Fig. 6.30 Solar radiation (Langleys) and hours of sunshine Christchurch Airport.

autumn are more rapid than the increases in spring.

(h) Distribution and seasonal variation of Zediloma subrostrata

The total biomass and number of Zediloma subrostrata individuals (Fig. 6.31) show a marked decrease from June to October. For the rest of the period, the levels are notably higher but fluctuate considerably from month to month.

As shown on Table 6.11, there is marked variation between and within areas. The levels at A, B and F are very much higher than those at C, D and E. The mean size of individual Z. subrostrata also differs between areas. The animals at A and C are normally smaller than at the other sampling sites. The size of individuals also tends to decrease in winter (Table 6.12).

The results of a survey of Zediloma by Kilner (1969) are given in Fig. 6.32 which indicate a more restricted distribution than found in this present study. As shown on Fig. 6.33, Kilner recorded maximum numbers of Zediloma in association with 25-50% cover of Ulva. Kilner also surveyed the Amphibola crenata but as this snail does not feed on the macroscopic algae it will not be discussed here.

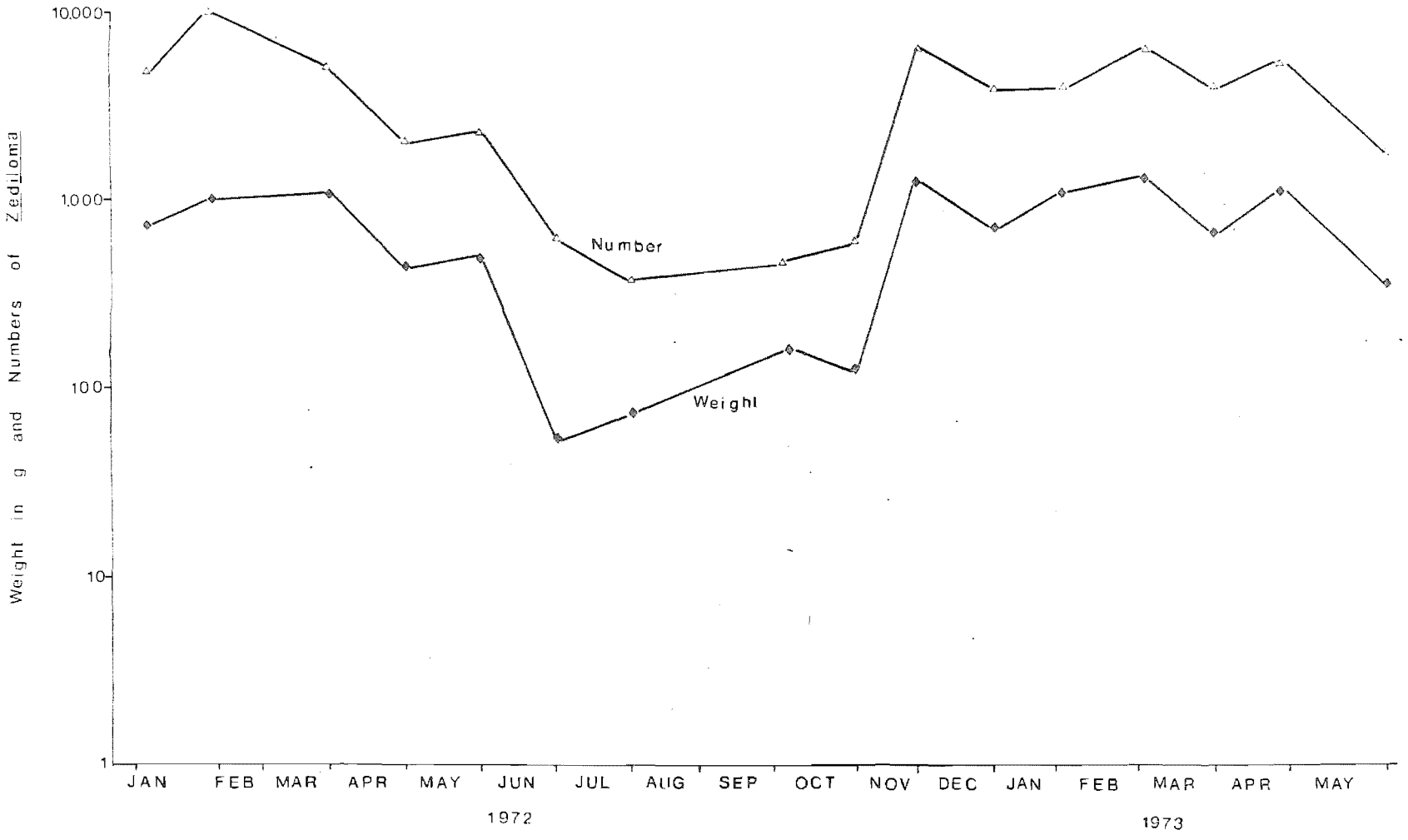


Fig 6·31 Zediloma subrostrata numbers and weight in g m

Table 6.11 Mean density of Z. subrostrata g/m^2

Month	SAMPLE AREA A Mean	A S.D.	B Mean	B S.D.	C Mean	C S.D.	D Mean	D S.D.	E Mean	E S.D.	F Mean	F S.D.
1972												
Jan.	163	51	65	53	2	0.16	68	36	7.0	6	N.S.	
Feb.	228	96	60	48	3	0.2	34	9	10.4	9	N.S.	
March	165	100	34	31	10	7.0	53	14	3.6	3	N.S.	
April	63	27	70	48	3	2.1	15	6	0.2	0.06	N.S.	
May	72	59	35	32	-	-	8	-	0.1	1.0	N.S.	
June	50	-	3	-	-	-	-	-	-	-	N.S.	
July	36	-	36	-	-	-	-	-	-	-	N.S.	
Aug.	-	-	-	-	-	-	-	-	-	-	N.S.	
Sept.	14	-	14.3	-	-	-	-	-	-	-	N.S.	
Oct.	57	-	-	-	-	-	-	-	-	-	N.S.	
Nov.	222	150	28	16	N.S.	-	15.7	6.3	2.6	2.0	172	83
Dec.	121	46	25	18	N.S.	-	7.7	4.0	0.5	0.4	257	102
1973												
Jan.	215	126	33	24	N.S.	-	5.3	3.0	1.2	0.8	173	33
Feb.	131	128	113	22	N.S.	-	2.9	1.5	1.1	0.9	136	100
March	92	48	43	32	N.S.	-	0.4	0.3	1.0	0.7	121	50
April	178	-	40	31	N.S.	-	0.2	0.1	4.0	5	259	130
May	28	20	43	29	N.S.	-	0.1	0.1	5.2	-	267	139

N.S. = No sample

S.D. = Standard deviation

Table 6.12 Mean biomass in g of Z. subrostrata

Month	SAMPLE AREA A	B	C	D	E	F
Jan. 1972	0.22	0.30	0.16	0.40	0.35	N.S.
Feb. "	0.23	0.27	0.14	0.50	0.29	N.S.
March "	0.16	0.37	0.50	0.28	0.56	N.S.
April "	0.13	0.48	0.10	0.17	0.18	N.S.
May "	0.17	0.57	0	0	0	N.S.
June "	0.15	0.23	0	0	0	N.S.
July "	0.15	0.10	0	0	0	N.S.
Aug. "	0	0	0	0	0	N.S.
Sept. "	0.24	0.32	0	0	0	N.S.
Oct. "	0.21	0	0	0	0.19	N.S.
Nov. "	0.17	0.41	N.S.	0.15	0.20	0.41
Dec. "	0.13	0.65	N.S.	0.27	0.35	0.20
Jan. 1973	0.25	0.41	N.S.	0.22	0.27	0.24
Feb. "	0.13	0.54	N.S.	0.25	0.38	0.16
March "	0.16	0.19	N.S.	0.18	0.26	0.15
April "	0.18	0.28	N.S.	0.14	0.63	0.20
May "	0.12	0.36	N.S.	0.06	1.20	0.25

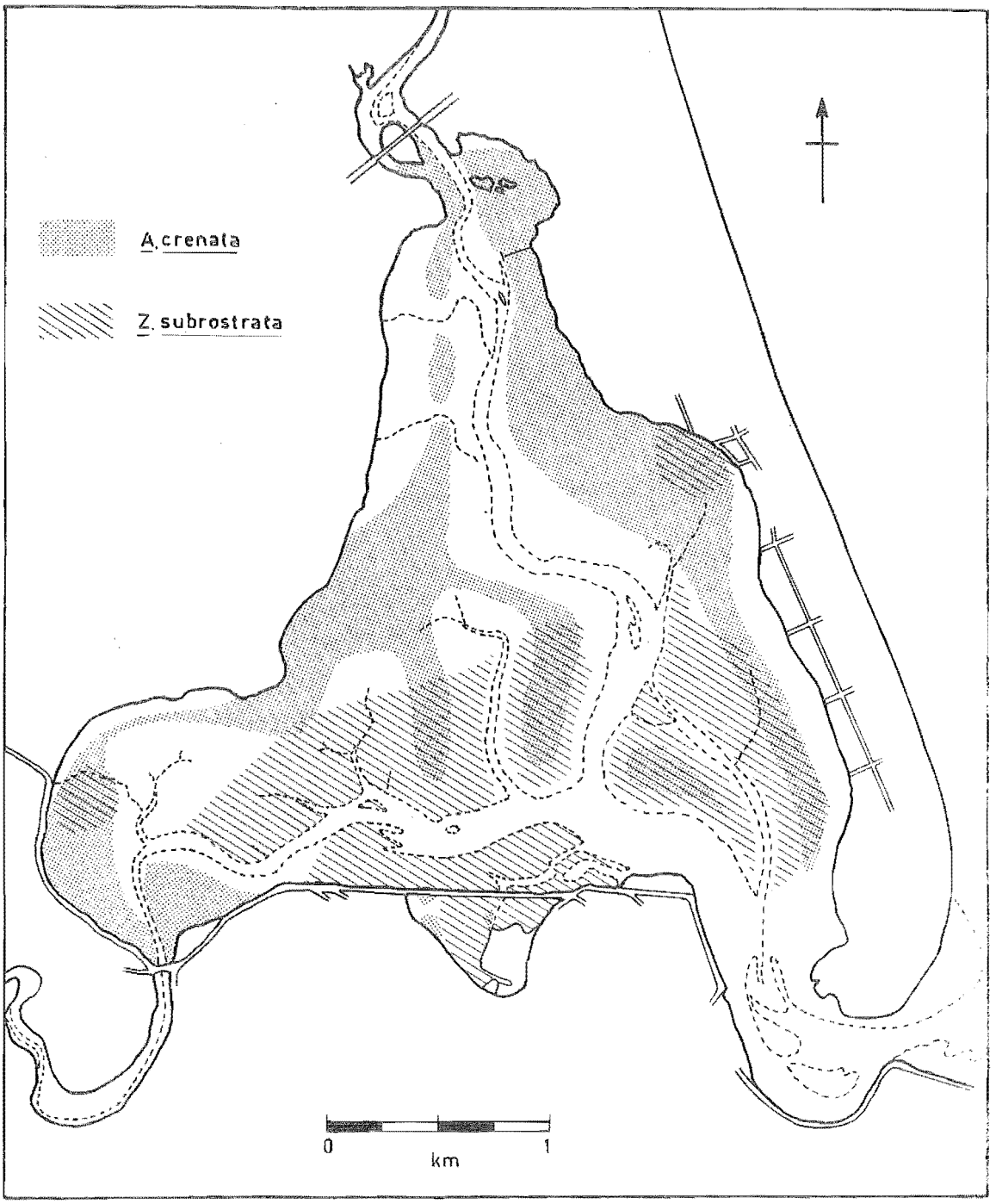


Fig. 6.32 Distribution of *Amphibola crenata* and *Zediloma subrostrata* in May, 1969 (after Kilner, 1969).

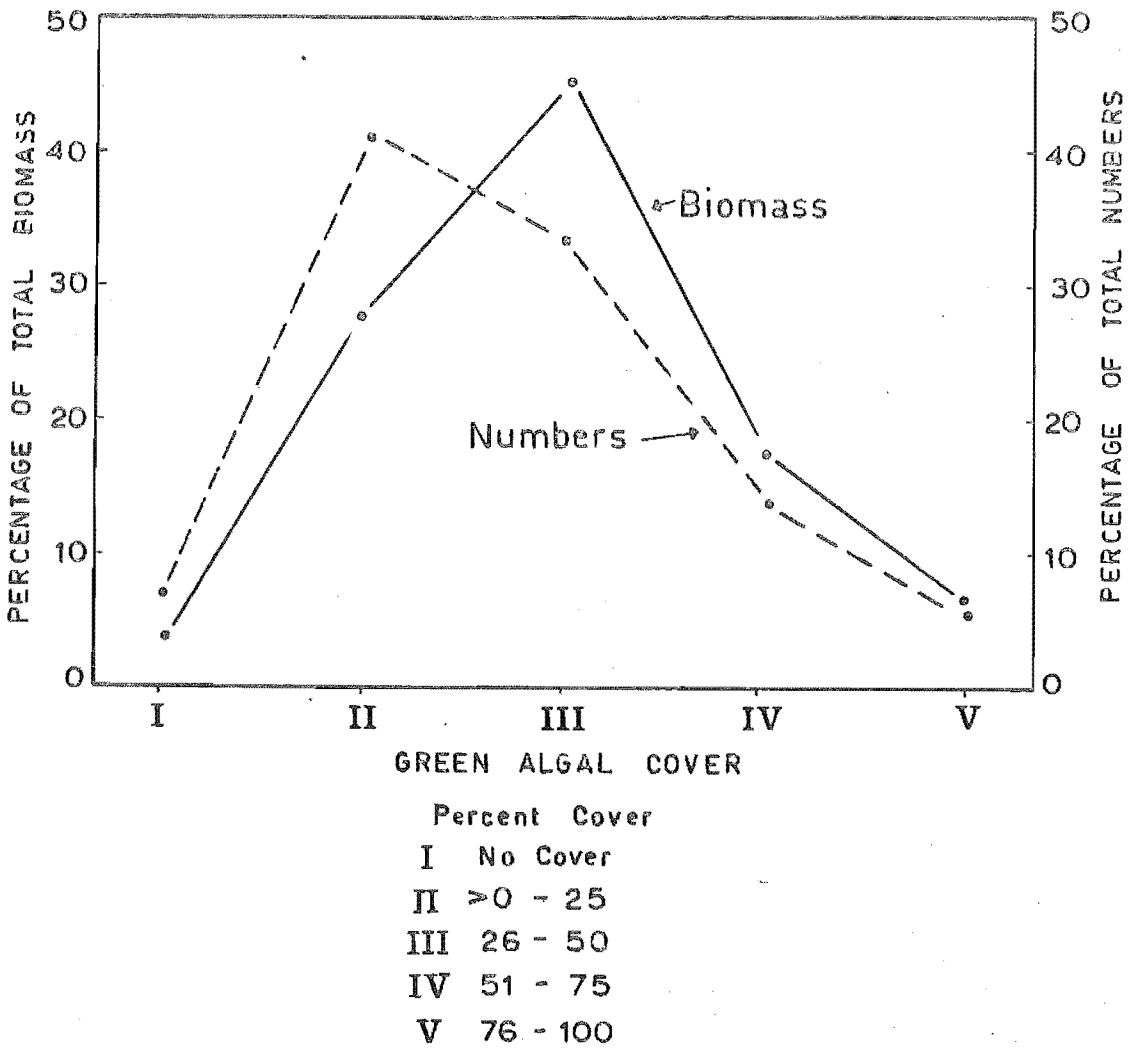


Fig. 6.33 Relationship between Zediloma subrostrata density and green algal cover (from Kilner, 1969).

3) RESULTS OF EXPERIMENT FIELD WORK FEBRUARY 1973 to MAY 1973

As mentioned in Chapter V, only two of the nine frames set out to enclose the quadrats retained the original algal population. Unfortunately, these two frames did not succeed in restricting Zediloma movement into the parts of the quadrats from which they had been removed at the beginning of the experiment. Although the population remained lower in the formerly Zediloma-free parts of the quadrats, the reduction in numbers is generally small in comparison with the variability of the Zediloma population as a whole. It is therefore not

possible to extrapolate with confidence from the enclosed quadrats to the general field situation with regard to the effect of grazing but enclosure has a marked effect on standing crop.

The results are shown in Plates 6.16 to 6.18 and summarised in Tables 6.13 and 6.14. It is assumed that the initial standing crop was similar for the enclosed and open quadrats of each area, as homogenous areas were chosen for the experiments.

The per cent cover in the enclosed quadrats increased noticeably during the experiment, especially at area F (Plate 6.18). The standing crop at the end of the experiment was also higher than that found in the adjacent open quadrats at the beginning of the experiment.

The density of area F as a whole was 13 grams per metre square with confidence limit of plus or minus 4 grams (see Fig. 6.13). The enclosures at area F had 178 grams per metre square in the Zediloma removed part and 76 grams per metre square in the Zediloma left part. The contrast was even greater for area B, the enclosure having a standing crop over tenfold greater than the upper limit for the area as a whole.

Table 6.13 Experimental field results Area B

AREA B g/m^2 of algae and Zediloma

	ENCLOSED AREA				OPEN AREA	
	Zediloma removed		Zediloma left		Zediloma left	
	Algae	Zediloma	Algae	Zediloma	Algae	Zediloma
Feb.		36			110	39
April	236	66	209	57	40	4.8

Table 6.14 Experimental field results Area F

AREA F g/m^2 of algae and Zediloma

	ENCLOSED AREA				OPEN AREA	
	Zediloma removed		Zediloma left		Zediloma left	
	Algae	Zediloma	Algae	Zediloma	Algae	Zediloma
Feb.				36	22	39
April	178	185	76	300	8.0	8.8

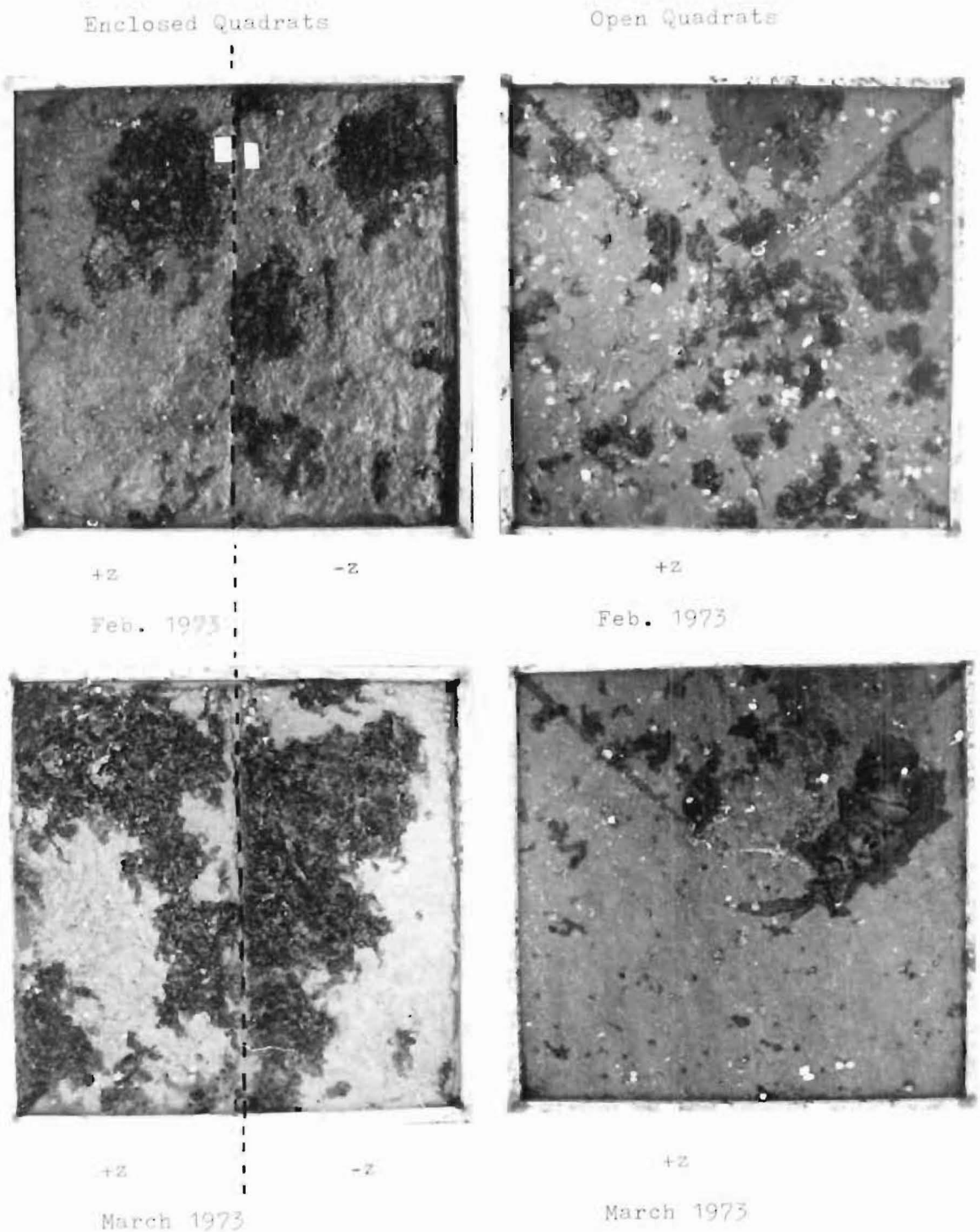


Plate 6.16 Enclosed and open quadrats at area A. +z = Zediloma left -z = Zediloma removed. ---=position of partition.

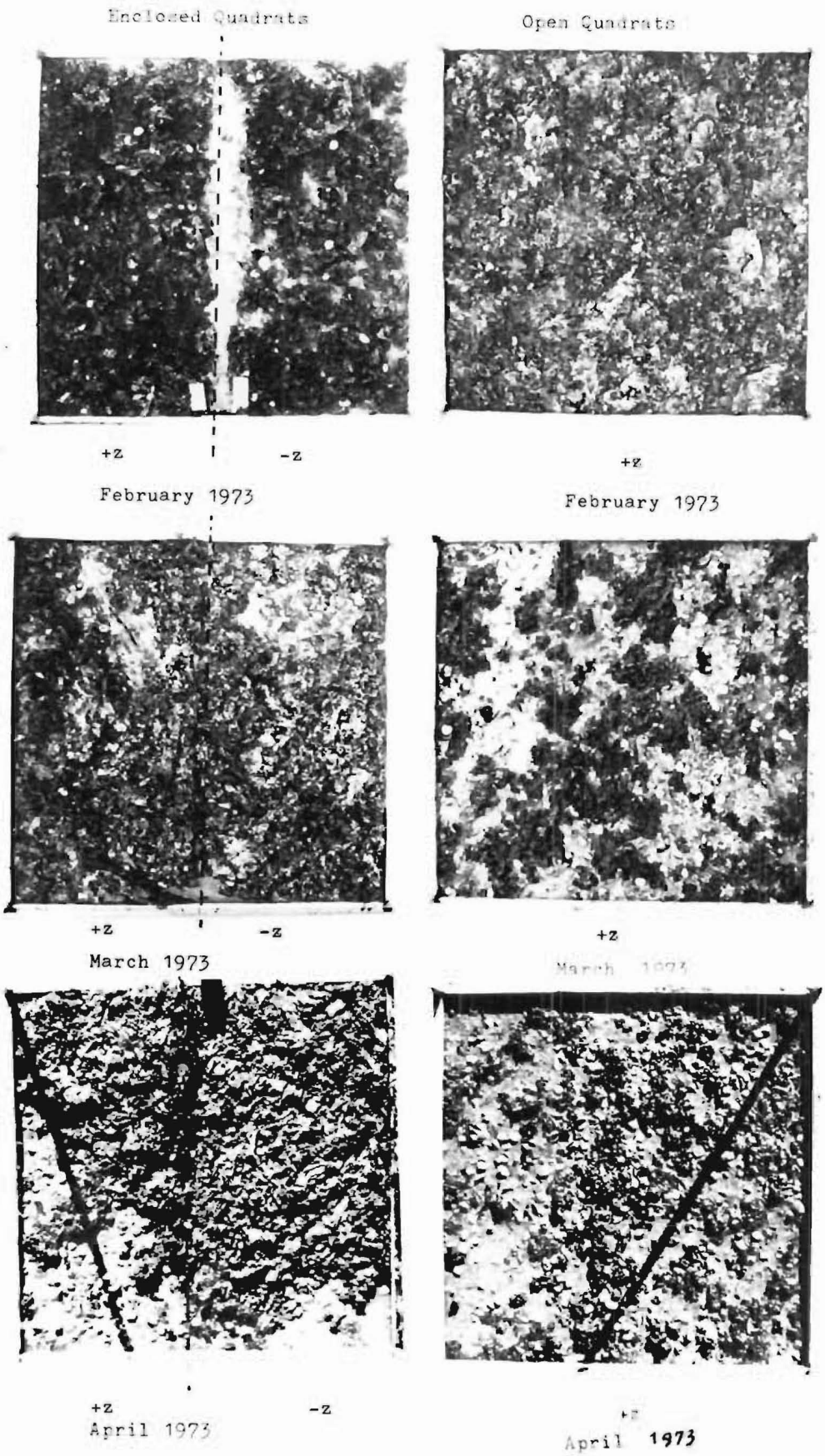
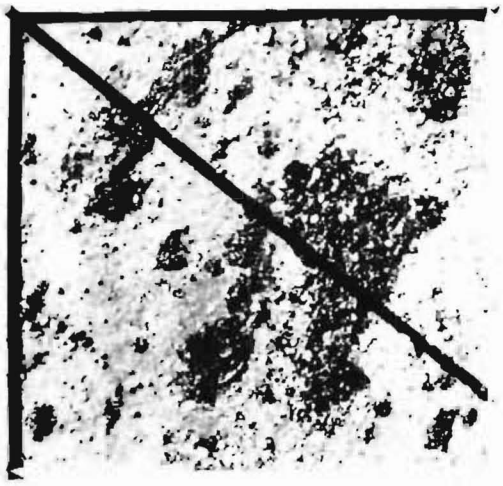


Plate 6.17 Enclosed and open quadrats, area B, see plate 6.16 for key.

Enclosed Quadrats

+z -z

Open Quadrats



+z

February 1973

+z -z

February 1973

+z

March 1973

April 1973

March 1973

April 1973

Plate 6.18 Enclosed and open quadrats, Area F. see plate 6.16 for key

CHAPTER VII

DISCUSSION OF FIELD RESULTS

1) INTRODUCTION

The field study clearly places Ulva lactuca as the dominant alga on the Estuary while Enteromorpha ramulosa, which was the dominant alga found by Cameron in January 1969, was of relatively minor importance during this study, even at its peak in summer. Gracilaria secundata was ecologically the least important, as has been shown in the previous surveys. Since Ulva lactuca is the most significant alga, the following discussion relates to this species.

The field results clearly point to the importance of the drift population of Ulva lactuca, especially in summer when standing crop levels are highest. This agrees with the observations of Bruce (1953) and Rosenberg (1963). It was most noticeable in the 1971/72 summer when regrowth occurred from the drifts which persisted throughout the previous winter. The smaller standing crop in the 1972/73 summer can be related to the absence of the drifts during the winter of 1972. Regeneration in spring was therefore entirely from growth and reproduction by zoospores and gametes from the attached plants. The drift again resumed its position of importance as detachment and fragmentation of the attached population contributed to the drift population.

Although drift and attached algae will both be responding to the general growth factors, some factors will have a greater effect on one or the other, so that these factors are best discussed separately for the drift and attached populations.

2) DISTRIBUTION OF DRIFT: *Ulva lactuca*

The detached plants are obviously at the mercy of the currents and will therefore only accumulate in areas of low current velocities. This will occur in depressions on the mudflats and in the backwater bays north of the Avon and Heathcote rivers, due to the current pattern as shown on Fig. 6.21. Fig. 6.22 indicates that the current velocities are higher on the flood tide than on the ebb tide. This will produce an east to west drift, reducing the standing crop in the east and central regions and increasing it in the western regions. This is reflected in Fig. 6.14 where the drift algae disappeared from areas A and B in March and April 1972, while at the same time areas C and D to the west had increased in algal standing crop and retained high densities until June and July, respectively. The enclosed quadrats at A, B and F in March and April 1973 also indicate that the currents redistribute much of the algae produced in these areas.

The sudden disappearance of drift plants in 1972 was associated with stormy weather in June and July, which produced river flooding and relatively strong wave action on the Estuary. Large quantities of algae were left stranded above the high tide mark; the remainder was presumably washed out to sea.

The currents will also affect the attached standing crop levels by increasing detachment and fragmentation. The results from enclosed quadrats and the rapid development of a drift population in the spring of 1972 indicate a continual depletion of the attached standing crop by current action. This will have the greatest effect in areas of high current velocities such as those in or near the main channels and in some areas the currents may be strong enough to exclude attached algae. In most cases, however, it will affect the standing crop level rather than distribution of the attached algae.

3) DISTRIBUTION OF ATTACHED PLANTS

The maximum frequency (number of plants per area) of attached algae is limited by the availability of suitable attachment sites. The most important settlement substrates are provided by the living and dead shells of the bivalves, Chione stutchburyi, Amphidesma australe australe and Macomona liliana. The bivalves are concentrated into beds at areas A, B and F and in the shallow channels and depressions in the eastern half of the Estuary. The areas to the west lack any firm substrates, with the exception of a shell bed south of the oxidation ponds which was uncovered in the July storms of 1972. These areas, which have very few bivalves, correspond to the above 60% silt clay fraction category on Fig. 6.15. The remainder of the Estuary has relatively sparse settlement sites.

Areas with apparently suitable substrates do not always have high algal densities, for example, a number on shell beds

in the eastern regions such as transect VI and quadrat (2) (see Fig. 3.1) lack a significant algal population. This may be due to the instability of the sandy substrate in these regions. The two foot marker pegs in these areas were often buried or almost completely exposed as a result of substrate movement. Any algae which settled on the shells would therefore be subject to continual abrasion from sand particles.

The central and eastern regions of the Estuary and the high tide zone also have a number of suitable substrates which are not completely taken up by the algae in summer. In contrast, all the available sites at areas A, B and F are fully utilised.

The density of the algae is also affected by the size of individual thalli. The algae at areas A, B and F are not only more frequent, but also have a larger thallus size than the regions in the central and north-eastern regions. This suggests that growth conditions are more favourable in these areas. This aspect will be discussed in the following sections.

4) FACTORS AFFECTING BOTH ATTACHED AND DRIFT PLANTS

(a) Exposure time

As Ulva lactuca has no obvious means of resisting desiccation, it is likely to be adversely affected by long periods of exposure. The exposure pattern, as shown on Fig. 6.20, places all the major concentrations of Ulva in the mid-tide zone. The lack of plants in the low-tide channels is probably related to the higher current flow, substrate instability and the low salinities. The long exposure periods

in the central regions and on Brighton Spit offer a logical explanation for the lower algal densities. Drift algae found in these areas usually show signs of stress or decay. Sample area E in the eastern region which had consistently had lower algal densities than areas A, B and F is also exposed longer. The areas submerged for longer periods would favour growth by providing less stress and a longer period of growth per tidal cycle. Johnson et al (1974) showed that Ulva expansa quickly lost its photosynthetic capacity in air and this may also occur in U. lactuca.

(b) Salinity

The effects of salinity have not been studied in detail in this investigation but the results of the culture experiment described in Chapter V indicate that salinities below 15°/oo are unfavourable for growth. This is similar to the results reported by Moshen et al (1972) for Ulva fasciata which indicated that the optimum was 25°/oo with little growth below 20°/oo and that reproduction was completely inhibited at 15°/oo. The intolerance to low salinity, therefore, explains the lack of Ulva in the low tide channels and in the lower reaches of the rivers. Elsewhere on the Estuary, salinity is unlikely to have a marked effect on distribution or productivity.

(c) Grazing pressure

The epibenthic gastropod Zediloma subrostrata is very common in some parts of the Estuary and is frequently seen feeding on Ulva lactuca. The thalli are often perforated and the margins show the effect of grazing.

The distribution of Zediloma as described by Kilner

(1969), (Fig. 6.32) shows a concentration in the central and on southern regions. Knox and Kilner (1973) suggest that an extension towards the rivers and the western shore is limited by low salinity.

The Zediloma distribution found during this study extends further towards the river mouths than recorded by Kilner, as indicated by the large biomass at area B. The marginal areas tend to have larger animals, as shown in Table 6.9, which may be more tolerant to low salinities. Kilner (1969) found that the Zediloma biomass was highest in the 26-50% algal cover category, but was lower in the very dense or very sparse cover categories (Fig. 6.33).

The results from this study do not indicate quite the same relationship. The sample areas with high algal densities within the range of Zediloma also have high Zediloma biomass. The pattern of seasonal change is similar for Zediloma and for the algae (Figs 6.14 and 6.31). However, this general relationship which holds between areas does not hold for individual samples within an area: when the Zediloma biomass is plotted against the algal density for each sample in a scatter diagram there is no discernable pattern. High algal densities usually have moderately to high Zediloma populations, but equally high Zediloma biomass also occurs at sites with very few algae.

Some of this variation could be expected. If it is assumed that the U. lactuca is a major food source for Zediloma, they will be attracted by high U. lactuca densities and will develop faster in these areas than in areas with little Ulva, producing a positive correlation between algal and Zediloma density. If the increase in Zediloma was such

that the removal of algae by grazing exceeded the rate of productivity, the correlation would tend towards the negative. This would explain the general relationship between areas and the variation found for individual samples within areas.

The enclosed quadrats designed to test this relationship were not entirely successful as Zediloma were able to invade the cleared areas. The results did suggest, however, that the high algal density within the enclosures attracted high Zediloma densities. Table 6.14 shows that the enclosed area at F contained 185 grams Zediloma, while the biomass on an open quadrat immediately adjacent to the enclosed quadrats was 9 grams (Fig. 6.13). The higher Zediloma figures in the enclosure from which they were not removed may also explain the lower algal density in that half of the frame.

As Zediloma are absent from the main areas of drift algae to the west of the Estuary, the lack of grazing pressure may accentuate the differences between these areas and the areas of drift to the east, which is already established by the current pattern. The results from the enclosed quadrats tentatively suggest that enclosure has a greater effect than removal of the Zediloma on standing crop, but the absence of grazing may be important in allowing the drifts to persist in the western areas throughout the winter (subject to wave conditions), whereas the drift to the east always disappears in winter.

The impounded area of McCormack's Bay has a very large population of Zeacumantus subcarinatus, a small epibenthic gastropod which feeds on U. lactuca and Chaetomorpha linum. Graham (1969) recorded densities of 1200 grams wet weight of

Zeacumantus per square metre. Almost every thallus in this region shows signs of grazing. Very high algal standing crop is maintained here despite the very heavy grazing pressure. This supports the suggestion that Zediloma may not have a major effect on Ulva standing crop in the Estuary.

(d) Effect of nutrients on standing crop levels

The areas with the most favourable nutrient levels could be expected to have high productivity and, therefore, a high standing crop, although there are difficulties in examining this relationship in the field.

Considerable fluctuations in nutrient levels according to the state of the tide are a major problem, the levels varying according to the proportion of the high nutrient sewage and river water to the low nutrient sea water. The levels are also affected by wind conditions which can alter the pattern of dispersal of the sewage effluent.

Also there is a lack of information on the role of the sediments in the nutrient dynamics of the Estuary. It is clear from Figures 6.16 to 6.19 that the fine sediments in the western regions form a considerable reserve of nutrients. The close correlation between the areas of high nutrient sediments and the pattern of effluent dispersal leaves little doubt as to the origin of these elements. The sediment/water interchange appears to depend on three factors: the relative concentration in the interstitial water and the overlying estuarine water (Syers et al, 1973), the redox potential of the surface layers of the sediments (Olson, 1958, 1964; Hynnes and Greib, 1970) and the turbulence of the water. Although there is no information available for this Estuary, the

results of Pomeroy et al (1965) may have some relevance. These authors found that the sediments were in equilibrium with water having phosphorus concentration of 0.022-0.028 grams per cubic metre. If a similar situation exists on the Estuary, it is possible that the sediments will release phosphorus at high tide when nutrients in the water are low, but adsorb nutrients at low tide when the levels in the water are high. Adsorption would also occur in areas directly affected by sewage discharge.

An additional complication is the number of forms in which the nutrients may be present and which are available to the algae to a varying degree. These forms are not stable and there can be transformations from one to another by chemical and biological activity. Phosphorus may occur in three forms: organic, inorganic bound to particles and inorganic dissolved (reactive). Only the latter is immediately available to the algae but the other two may break down to the dissolved form as the concentration in the water decreases. The molybdenum blue method for determining reactive phosphate may also detect some of the smaller organic phosphate (Rigler, 1968) but it is not known whether this form of phosphate is available to the algae. It is therefore possible that the molybdenum blue technique may over-estimate the available phosphate. The position for nitrogen is even more complicated as it occurs in four main forms: organic, ammoniacal, nitrate and nitrite, all of which may be utilised to some degree by the algae as will be discussed in Chapter VIII. These forms also appear to be less stable than those of phosphorus. The main nitrogen transformation on the Estuary is from the ammoniacal form to the organic as Wilkinson (1963) found that ammonia

formed 50% of the incoming nitrogen but only 2% of the outgoing nitrogen. Wilkinson's calculations indicated that the amount of organic nitrogen formed in the Estuary exceeded the supply of inorganic nitrogen from the sewage effluent and the rivers. This he regarded as "presumptive evidence that nitrogen fixing bacteria are active in the Estuary". Wilkinson, however, ignored a number of potential sources of nitrogen. Firstly, he assumed that the nitrogen entering in the sea water left unaltered on the next tide, whereas it is possible that some of it was converted to the organic form while in the Estuary. Secondly, the death and breakdown of the freshwater algae discharged along with the sewage effluent may also add to the organic nitrogen in the outgoing water. Thirdly, the sediments may contribute organic nitrogen as discussed above.

The nutrient levels at any one area or at any one time are, therefore, the result of a complex interaction of a number of factors making it difficult to obtain comparative data. The most reliable information available is in the results from the samples taken over the full tidal cycle (see Figs 6.25 and 6.26). Unfortunately, the limited time available and the lack of a reliable technique for measuring ammonia in waters of varying salinity restricted the analysis to nitrate nitrogen. This will lead to under-estimating the amount of nitrogen available to the algae, particularly in the areas affected by the sewage effluent. There are also no data on the levels in the tidal pools at low tide which may become more concentrated due to evaporation and leaching from the sediments.

From Figs 6.25 and 6.26 it is possible to compare the

nitrate and phosphorus levels between sites at various stages of the tide. The ranking of the areas is similar for phosphorus and nitrate, but there are smaller differences between areas for nitrate, especially at high tide. The ranking on the flood tide in ascending order is A, F, E, B, D, C, while on the ebb tide the order is A, F, C (E, B, D). The means from all samples are shown on Fig. 6.20 are ranked A, E, (B, F, D), C for nitrate and A, F, B, E, C, D for phosphorus. Very high levels were sometimes found at D when the sewage effluent was pushed south-west by easterly winds, resulting in a sharp increase in P and N and a decrease in salinity.

To assess the effect of variation in nutrient levels on productivity, comparisons can only be made between areas with significantly different nutrient levels, but with similar exposure times, grazing pressure and substrate availability. The only areas which may meet these criteria are area B and quadrat (1) (see Fig. 3.1 for locations). Quadrat (1) is on a small flat area adjacent to the river channels in Monck's Bay. As the water at this point is not affected to any degree by the rivers or sewage effluent, its nutrient levels are only slightly higher than for the coastal sea water. The comparison between area A and quadrat (1) is summarised in Table 7.1.

Table 7.1 Nutrient levels at area B and quadrat (1).

	Area B	Quadrat (1)
Nitrate concentration	0.14 g/m^3	0.02 g/m^3
Phosphorus	0.07 g/m^3	0.01 g/m^3
Zediloma	30 g/m^2	20 g/m^2
Algae	140 g/m^2	71 g/m^2

The concentration of nitrate and phosphorus is seven times higher at B than at (1), while the algae are twice as abundant in spite of the higher grazing pressure. Although these results are by no means conclusive, they suggest that the higher nutrient levels in the western regions may stimulate higher productivity.

From this discussion of the field results, it appears that the main factors affecting the distribution of Ulva lactuca are current patterns, exposure times and substrate availability. Grazing by Zediloma has a significant effect on standing crop abundance in some areas. Higher productivity as a result of higher nutrient levels may contribute to the abundance of algae in the western region of the Estuary. The effects of nutrients on growth has been investigated in the laboratory and will be discussed in Chapter VIII.

Although the comments in this chapter refer to U. lactuca, most could also apply to Enteromorpha and Gracilaria. The main difference is the smaller proportion of these algae found in the drifts. Both species have smaller thalli, which present less resistance to the currents and may therefore remain attached longer than U. lactuca.

5) SEASONAL VARIATION IN ALGAL STANDING CROP

All three species decrease in abundance in winter; this decline is most marked in Enteromorpha ramulosa. The environmental factors having the most noticeable seasonal fluctuation are meteorological variables and of these, light and temperature are likely to have the most effect on algal productivity.

The only data available on light conditions are from the Christchurch International Airport. As this is nine miles from the Estuary and subject to different cloud conditions, the levels of solar radiation cannot be assumed to be the same as at the Estuary, but the general pattern of change should be similar. This pattern in mean langleys per day generally is similar to that of standing crop changes, but is about one month out of phase (Fig. 6.33); that is, the peak solar radiation occurs one month prior to the standing crop peak. The correlation between solar radiation and algal standing crop is not consistent throughout the year. For instance, August and April are similar in solar radiation, but differ greatly in algal standing crop. The data available for McCormack's Bay also do not indicate a close relationship between solar radiation and algal standing crop as the area covered by the algae is relatively stable throughout the year. Cameron (1969) found maximum algal density in winter. As the solar radiation would be the same for all the Estuary, this factor does not explain the complete lack of algae on the main body of the Estuary during winter.

Day length or photoperiod may also affect seasonal growth patterns. An indication of photoperiod changes is given by the hours of sunshine recorded on Fig. 6.30. This pattern of change is similar to that for solar radiation but has less amplitude. The lack of a marked seasonal pattern in growth of the algae in McCormack's Bay would also suggest that photoperiod or sunshine hours may be of relatively little importance.

The temperature data being collected at the Estuary are more relevant than the solar radiation. Data from McCormack's

Bay also suggests that temperature may be of greater importance. Unpublished data collected by the members of the Zoology Department give the water temperature in McCormack's Bay as 2°C higher than the adjacent Estuary, which is confirmed by my own records. This may explain the high winter densities found in McCormack's Bay. This aspect will be discussed further in Chapter VIII.

The pattern of change in water and air temperature at the Estuary follows the same general trend as the algal densities. Temperature could be expected to have a positive correlation with photosynthetic rates, increases promoting growth until the optimum is reached. Above the optimum, respiration may be stimulated to a greater extent resulting in a decrease in net productivity.

To assess the effect of temperature, it is necessary to have a direct measure of productivity. As already mentioned, standing crop losses may occur through grazing and current action and the absolute level of standing crop is also affected by the size of the persistent winter population. In addition, the expansion of the populations may be restricted by a lack of settlement sites or areas in which the drift population can be trapped. As the winter population in 1972 was very small, the standing crop increases reflect productivity during this period. There would also be no restriction due to lack of space, particularly in early spring. The data from this period are, therefore, of greatest value when assessing the effect of temperature.

In a population lacking restrictions on growth, the standing crop increase should be exponential; that is, when plotted on a logarithmic scale against time, the values should

lie on a straight line, with the slope of the line indicating the rate of increase. Restrictions such as lack of space or nutrients and removal of standing crop will produce growth curves below those predicted for exponential growth. Sub-optimal temperature will affect the slope of the growth curve rather than its shape.

The only part of the standing crop curves for U. lactuca which approach a straight line (Figs 6.12, 6.13 and 6.14) lie between October and December. This period also has the fastest rate of increase. This suggests that the conditions were more suitable during this period and that there were fewer factors restricting the expansion of the population. For the attached population of U. lactuca, the shape of the curves is similar for areas A, B and F, but the curve for E is very much lower. This suggests that one or more growth factors were less favourable at E than at A, B and F. The results, as previously discussed, indicate exposure as a probable factor.

As substrate availability is unlikely to restrict expansion prior to October and as loss due to fragmentation and grazing would be relatively unimportant, the factor most likely to restrict growth rate during this period is temperature. The water temperature in August was $8^{o}C$, but increased sharply to $12^{o}C$ in October. There were further increases of $2^{o}C$ per month up to November and $1^{o}C$ per month to the maximum of $19^{o}C$ in February. The maximum rate of standing crop increases therefore occurred at temperatures between $14^{o}C$ and $17^{o}C$. The lower rate of increase in algal density after December may be the result of temperatures above optimum. Unfavourably high temperatures would have more effect in

McCormack's Bay, which may explain the reduced densities reported by Cameron in the 1968/69 summer. High atmospheric temperatures in summer may also accentuate the stress caused by exposure at low tide.

There are a number of other factors which may affect standing crop densities in summer. By December most of the attachment sites at A, B and F were occupied, limiting standing crop increases to growth of individual plants or the expansion of the drift population. From this point onwards, density of attached algae does not reflect the full growth potential. The large thalli common in December are more susceptible to fragmentation or detachment which would increase loss to the drift population. The density of the drift increased significantly between November and December. The drift, however, can also expand by increasing in area, reducing the value of density as a criterion of abundance. Density remained relatively stable after December, but Fig. 6.10 and Table 6.4 show that the area of drift algae increased significantly during this period and so did total standing crop.

The drift population may also be subject to space restriction as the depressions on the mudflats become full. This would increase the amount of algae washed out to sea or trapped above the high tide mark. The small areas of drift present in the 1972/73 summer, compared with previous summers, suggest that the drift population had considerable scope for expansion.

The loss of standing crop due to grazing will also grow in summer as the biomass of Zediloma increases.

The standing crop densities of Ulva decreased from

March to May 1973. Enteromorpha decreased from February while Gracilaria reached its peak in April. As the Zediloma population remained stable during this period, the decreases in Ulva and Enteromorpha reflect a real change in productivity, which is associated with a sharp drop in temperature.

The field results, as discussed above, point to temperature as the major factor in controlling seasonal growth patterns. Ulva and Gracilaria appear to be more tolerant to low temperatures than Enteromorpha. The prominence of Gracilaria in autumn may also be the result of reduced competition for attachment sites during this period.

In summer, limited attachment sites, increased grazing pressure and the dispersal of the drift plants restrict the expansion of the algal population.

Year to year variations

The major difference between 1971 and 1972 was the absence of the drift in the 1972 winter. This is reflected in the lower standing crop in the 1972/73 summer, despite the high growth rate in the spring of 1972.

From previous studies during the periods of low standing crop abundance (Rosenberg, 1962/63 and Webb, 1965), the drift also made up a relatively small proportion of the total population.

The highest standing crop occurred in 1968 and 1969 when Enteromorpha was an important element of the population. Table 6.6 confirms Cameron's observation that the Enteromorpha bloom was preceded by above average atmospheric temperatures. The association between above average standing crop and high summer temperatures supports the suggested importance of temperature as the main factor in the seasonal growth pattern.

CHAPTER VIII

LABORATORY STUDIES ON THE EFFECT OF NUTRIENTS AND TEMPERATURE ON ULVA LACTUCA

The aim of this part of the study was to investigate the effect of nutrient concentrations and temperature on Ulva lactuca by means of controlled experiments which eliminate interference from other factors complicating the field results.

1) EFFECT OF PHOSPHORUS AND NITROGEN ON GROWTH RATES

(a) Literature review

As discussed in the general introduction, it is widely agreed that phosphorus and nitrogen are the most important nutrients in eutrophic conditions. There is, however, no consensus as to the relative importance of these two elements or the concentrations which will stimulate algal growth. Much of the literature on this subject refers to planktonic species and the levels of nutrients which limit or promote "bloom", i.e. nuisance densities. This search for limiting factors or limiting concentrations follows the concept of Liebig's law of the minimum which was stated by Taylor (1934) as follows: "The growth and functioning of an organism is dependent upon the amount of the essential environmental factor presented to it in minimal quantity during the most critical season or years of the life cycle". Growth could therefore be restricted if a single nutrient were below the organism's requirements

irrespective of the levels of other nutrients. Goldman (1972), in a review of the limiting factor concept, noted that a single effect could result from several causes and that it is an over-simplification to apply this concept to natural multi-species plankton populations. This is supported by Cain (1944) who argues that as "physiological processes are multi-conditioned it is impossible to speak of one factor as being the cause of an observed effect". Although it is possible for a single factor to limit growth, the application of this concept requires a knowledge of the state of all other growth factors.

As phosphorus concentrations are generally low in natural waters during periods of excess growth, phosphorus is assumed by many workers to be the nutrient most in demand by the algae and the one most likely to limit further growth (Gerloof and Skoog, 1957; Goldman and Wetzel, 1963; Ketchum, 1939, 1954; Rigler, 1956; Saunders, 1957; Sawyer et al, 1944, 1954; Rodhe, 1948). Minimum phosphorus concentrations required for an algal bloom are given as 0.015 grams per cubic metre (Sawyer et al, 1944), 0.2 grams per cubic metre (Rodhe, 1948 for Ankistrodesmus falcatus) and 0.17 grams per cubic metre (Ketchum, 1954 for Nitzschia closterium). Supra-optimal phosphorus levels may also inhibit growth as reported by Chu (1948) for Asterionella formosa at levels above 8.9 grams per cubic metre. Although there are considerable interspecific differences, Ketchum (1939) and Goldberg et al (1951) conclude that phosphorus is not normally limiting at levels higher than 0.016 grams per cubic metre.

The levels quoted above are very much lower than those found in eutrophic waters, prompting many researchers to

regard nitrogen as the limiting factor in these habitats (Allen, 1955; Dugdale and Goering, 1957; Gerloof et al, 1950, 1952; Goldman, 1960; Staub, 1961; Talling, 1958, 1965; Kratze and Meyers, 1955; Gerloof and Skoog, 1957). A minimum level for a plankton bloom is given by Sawyer et al (1944) as 0.3 grams per cubic metre N. The position for nitrogen is complicated by the variety of sources and forms of nitrogen including fixation of atmospheric nitrogen by bacteria and blue-green algae. Sawyer and Ferullo (1961) regard phosphorus as the critical element in nitrogen fixation in the cyanophyta which supports their claim that phosphorus rather than nitrogen is most likely to limit growth.

The concentration of nitrogen in natural waters is usually higher than that of phosphorus. Redfield (1958) gives an atomic ratio of 15N:1P as typical for sea water as a whole. Knox and Kilner (1973) suggest that plants assimilate N and P at a ratio of 10:1. In sea water, therefore, phosphorus is more likely to be limiting. Drainage water tends to be high in phosphorus, thus lowering the N:P ratio in lakes and coastal waters. Ryther and Dunstan (1971) conclude that most coastal water has twice the amount of phosphorus as can be used by the algae present. Thus nitrogen would be the limiting factor in estuaries. Support for the N:P ratio as the critical "bloom" factor is given by Gerloof and Skoog (1957) and Goldman and Carter (1965).

In much of the literature discussed above it is assumed that the rate of assimilation of P and N is determined by the growth requirements of the organism and is constant within species. However, Carpenter and Guillard (1971) give evidence for physiological races of marine plankton adapted to high or

low nutrient levels. In addition, Kerr et al (1970), Goldberg et al (1951), Rodhe (1948), Fitzgerald (1965), Batterton and van Baalen (1958) report luxury uptake of phosphorus by phytoplankton. If the stored phosphate could be utilised when the concentration in the water decreases, then the growth rate could be independent of the immediate concentration in the water.

The responses to nutrient concentrations recorded for planktonic species may not be applicable to benthic species because of habitat differences; for example, the continual renewal of nutrients by tidal movements in the water surrounding estuarine algae may facilitate nutrient uptake. These algae may also have quite different nutrient requirements from those of the planktonic species. This is supported by the existing data for Ulva and Enteromorpha as discussed below.

Foster (1914) demonstrated that growth in Ulva lactuca was stimulated by urea and ammonium nitrate up to concentrations of 5 grams per cubic metre N and by acetamide up to 10 grams per cubic metre. Foster suggested that ability to utilise organic nitrogen may explain the association between abundant Ulva lactuca and sewage pollution reported by Letts and Richards (1911).

Andersson (1942, 1943) showed that nitrate was the preferred source of nitrogen for U. lactuca, Enteromorpha linza and E. intestinalis except at low concentrations while ammonium sulphate was toxic at levels above 1.0 grams per cubic metre N. E. linza was the least tolerant to high ammonia which, Andersson noted, could be related to less polluted water in which this species grew. The optimum alkali phosphate

concentration was 1 gram per cubic metre P for U. lactuca and E. linza and 0.5 gram per cubic metre P for E. intestinalis with growth impeded at higher concentrations.

Andersson's results were confirmed by A. Kylin (1944, 1945) and H. Kylin (1943) who also demonstrated that other ammonium salts, esters, amides and amino acids could be used as a nitrogen source by Ulva lactuca.

Waite and Mitchell (1972b) found that maximum stimulation of carbon assimilation in U. lactuca occurred at 0.5 gram per cubic metre P (phosphate phosphorus) and 0.6 gram per cubic metre N (ammonium nitrogen). Higher levels of nitrogen impeded growth. Additions of either phosphate or ammonium stimulated growth so that it is the absolute concentration rather than the N:P ratio which is important for this species. Waite and Mitchell (1972b) regard the single limiting nutrient concept as inadequate to explain these results and suggest the Baule-Mitcherlich limiting factor equation as proposed by Baule (1918) and modified by Verduin (1964) as a more useful model. This equation will be discussed in Chapter IX.

Table 8.1 Summary of optimum levels of P and N from different sources in g/m^3 of P or N

Author	Sodium nitrate	Sodium nitrite	Ammonium nitrate	Reactive phosphate
Foster (1914)	-	-	0.5	-
Andersson (1943)	0.82	0.44	0.35	1.0
Kylin (1945)	0.82	0.44	0.35	-
Waite and Mitchell (1972b)	-	-	0.6	0.5

The luxury uptake of nutrients recorded for planktonic species also occurs in benthic algae and macrophytes. Fitzgerald (1969, 1970) argues that the amount of phosphorus and nitrogen stored in the tissues and the activity of associated enzymes can be used as an indicator of the nutrient status of the water; that is, plants growing in water with a nutrient at levels above the optimum for that species will accumulate it in their tissues. This is more meaningful than the absolute concentration in the water as it relates the nutrient status of the water to the requirements of the species growing in it. To interpret the significance of a particular level of a nutrient in the tissues, it is necessary to make determinations on plants from waters with varying nutrient levels. The data available for Ulva and Enteromorpha are summarised in Table 8.2.

These results clearly indicate that Ulva and Enteromorpha in polluted localities have higher nitrogen levels in the thallus than those in unpolluted areas. The sulphur concentration in polluted waters is also higher than in 'clean' waters but Letts and Richards (1911) and Bruce (1953) found no difference in the sulphur content of the algae. This is understandable as sulphur is not a major nutrient for them.

(b) Laboratory materials and methods

All experiments were carried out under controlled lighting and temperature.

Lighting: Light was provided from a bank of six cool white and six 'gro-lux' fluorescent tubes arranged alternately above the culture vessels. The 'gro-lux' lamps were used to

Table 8.2 Nitrogen content of *Ulva* and *Enteromorpha* from clean and polluted waters as % N

Author	Clean water	Polluted water	Polluted / Clean
A. *Ulva*			
Letts and Richards (1911) Great Britain	1.73 1.13 1.18	3.9 3.7 3.7	2.8:1
	1.35 (Mean)	3.76	
Bruce (1953) New Zealand	2.15+ 2.13+	3.9 4.0 3.8 3.9 3.9	1.82:1
	2.14 (Mean)	3.9	
Wilkinson (1963) New Zealand	2.9+	4.0	1.38:1
Cameron (1968) New Zealand	1.1	3.2	2.9:1
Subaramaih and Palvelk (1966) India	1.76	3.4	2:1
B. *Enteromorpha*			
Wilkinson (1963)	-	4.1	-
Letts and Richards (1911)	1.1	4.0	3.62:1

(+ some pollution from sewage)

obtain a more natural spectrum through their higher output in the red region than obtained from 'cool white' lamps. The light intensity was 60 lumen per square foot throughout the study. This was the maximum level practical with the equipment available and in a preliminary experiment gave better growth than lower intensities. To avoid light gradients, the outmost 20 centimetres at each end and the outmost 10 centimetres at each side were not used. Within the experimental area there was no light gradient detectable with an E.E.L."light Master" photometer. The photoperiod was controlled by a time switch which also operated two fans to cool the lamps.

Temperature control: The experiments were carried out in a room in which ambient temperature was kept at between 13^{o} and $17^{o}C$. This was more stable than normal room temperature but not adequate for controlled experiments. Therefore, further measures had to be taken.

For experiments I and II the temperature of the culture medium was controlled by placing the culture vessels in a water bath under the fluorescent tubes. The water was circulated from this water bath through 40 feet of 3/8 inch stainless steel heat exchange coils placed in a second bath. In this the temperature was controlled by a modified domestic refrigerator and a thermostatically controlled heating and stirring unit. When functioning, this system controlled the temperature to plus or minus $1^{o}C$ but, unfortunately, was not reliable as the results from experiment I illustrate. Subsequently, more reliable equipment was purchased and used in a redesigned control unit, represented in Fig. 8.1. This system used larger volumes of cooling water, better

circulation and more accurate temperature control and was successful in providing stable temperatures.

Redesigned temperature control unit: The water bath containing the culture vessels was 243 centimetres long, 60 centimetres wide and 30 centimetres high. A false bottom positioned 2 centimetres above the actual bottom stretched the full width of the tank but stopped 14 centimetres from each end. The coil from a Grant cc20" cooling unit was placed underneath the false bottom. The water was circulated over the cooling coil by two "Little Giant" 2E N.Y. submersible pumps at one end of the tank, each of which was capable of pumping 300 gallons per hour to a height of one foot. At the opposite end of the bath was a "Grant" S.U.2P pumping temperature unit and the stirring temperature unit from the original system (see Fig. 8.1 for diagram). The culture vessels were set on a wire mesh in the main body of the tank. The height of the frame could be adjusted to suit the size of the vessels.

Culture vessels: For experiments I to IV the culture vessels were narrow glass tanks 26 centimetres high, 20 centimetres long and 7 centimetres wide. They had the advantage of a large volume (3.5 l), but occupied a relatively small space under the lights. The experimental algal material in the form of small discs of thallus was suspended within the tanks on plastic frames covered with nylon. Each frame was subdivided into four compartments by nylon netting partitions and the frames were placed 1 centimetre below the surface of the culture medium. In this way, individual discs could be followed throughout the experiment and each was subject to the same light intensities. Air was bubbled into each tank to

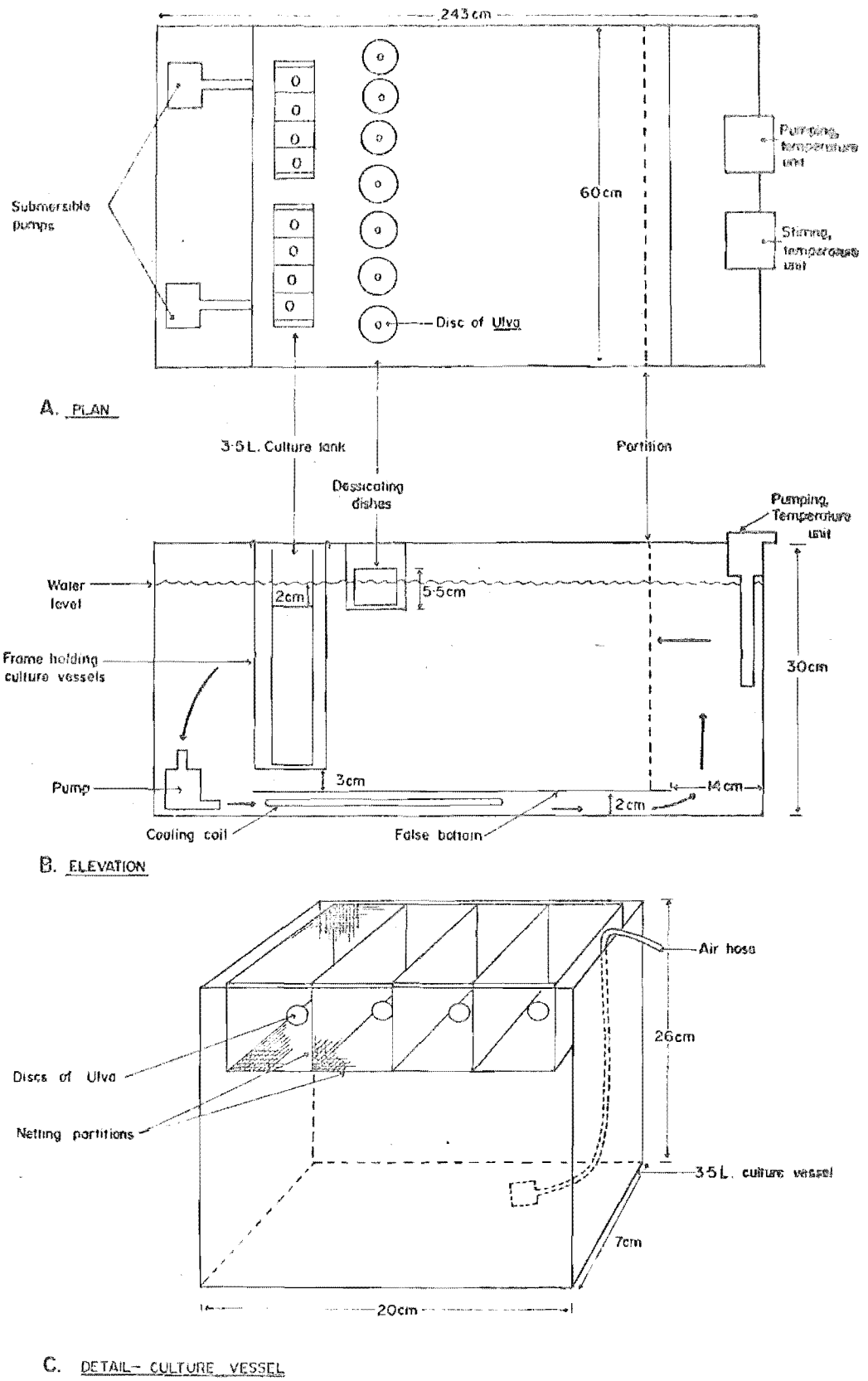


Fig 8.1. Temperature Control Unit as used in Experiments III to VI with detail of culture vessels.

maintain the CO2 concentration and provide mixing.

For experiments V and VI the 3.5 tanks were replaced by desiccating dishes 5.5 centimetres high and 9 centimetres in diameter. As these dishes contained only 200 millilitres of medium (1 centimetre deep), it was assumed that surface diffusion would maintain an adequate CO2 concentration.

All glassware was washed in hot water and rinsed thoroughly in distilled water before use in the laboratory experiments.

Preparation of culture media: The stock solution consisted of 80% natural sea water and 20% distilled water resulting in a salinity of 26% which is typical for the Estuary. Unless otherwise stated, the stock was filtered through Whatman number one filter paper and autoclaved at 15 pounds per square inch for twenty minutes in batches of 750 millilitres; larger volumes increased the problem of precipitates forming during heating. The enrichments detailed below were made up in sterile distilled water and added to the stock after autoclaving.

In experiments I and II the ASW8 medium as described by Provasoli (1957) was chosen as it is one of the more complete media in which this species has been previously cultured.

ASW8

Element	Quantity per of medium
Fe (as Cl)	0.5 mg
P11 metal mix	3 ml
S3 vitamin mix	0.5 ml
Vitamin B12	0.01 ug
Tris (Hydroxymethyl) amino-methane buffer	1 g

P11 Metal mix	mg per ml
Fe (as Cl)	0.01
B (as H_3BO_3)	0.2
Mn (as Cl)	0.04
Co (as Cl)	0.001
Zn (as Cl)	0.005
E.D.T.A. (Ethylenediamine -Tetraacetic Acid)	1.0

S3 Vitamin mix	Concentration per ml
Thiamine HCl	0.05 mg
Nicotinic acid	0.01 mg
Ca pantothenate	0.01 mg
para-aminobenzoic acid	1.0 ug
Biotin	0.1 ug
Inositol	0.5 mg
Folic acid	0.2 ug
Thymine	0.3 mg

The tris-buffer was adjusted with HCl to a pH of 7.0 which, although more acid than recommended in the literature, is the level typical of the Estuary. E.D.T.A. was used as a solubilising metal buffer or chelating agent. Germanium Dioxide was also added at 10 grams per cubic metre to inhibit diatom growth as recommended by Lewin (1966). Phosphorus was added as K_2HPO_4 and nitrogen added as $NaNO_3$ as required for each experiment. For experiment III and subsequent experiments the P11 metal mix and the S3 Vitamin mix of the ASW8 medium was replaced by the enrichment recommended by von Stosch (1957). This medium is simpler than the ASW8 but equally effective in this case.

von Stosch medium

Element	Quantity/l
$FeSO_4$ $7H_2O$	278 ug
$MnCl_2$ $4H_2O$	19.8 ug
Na_2 E.D.T.A. 2 H_2O	3.7 mg
Thiamine Hcl	0.2 mg
Biotin	1.0 ug
Vitamin B12	1.0 ug

Measurement of growth: The criterion used to assess growth was the increase in area of discs cut by a corer from the expanded region of adult thalli with fronds 20 centimetres in length. This technique was successfully used by Rhyne and Hommersand (1971) to measure growth in Ulva curvata and gave satisfactory results in the present study. The growth in the expanded part of the thallus appears to be diffuse with no specialised growing regions. This region is also of uniform thickness so that the dry weight should change according to area. In order to retain uniformity within each experiment, a single plant was used for each experiment except in experiment V where two plants were required to obtain sufficient discs of uniform thickness.

While in experiment I increase in area was estimated from micrometer measurements of the diameter, in subsequent experiments the discs were photographed and the area determined with a planimeter. This provided a permanent record of the size and shape and it was more convenient to measure from the photographs than from the discs themselves. The photographs were made by laying the discs on a sheet of glass and covering them with thin plastic over which unexposed

photographic paper was spread. The glass and paper were then inverted and exposed under a photographic enlarger. After the discs had been arranged on the glass plate, operations were carried out under a "safe" light to avoid exposing the photographic paper. The plastic was employed to avoid contamination by the photographic emulsion as the discs were measured a number of times during some of the experiments.

Selection of experiment plants: As previously mentioned, there is evidence of luxury storage of nitrogen and perhaps of phosphorus in the Ulva from the Estuary. As this could influence the growth responses in the laboratory, it was necessary to determine the phosphorus levels in the thallus before embarking on the experiments.

The phosphorus level in the thallus of plants from areas A, B and C was determined by the method described by Fitzgerald (1969). Approximately 0.1 gram of Ulva lactuca was placed in 40 millilitres of distilled water in a boiling tube which was left in a boiling water bath for 60 minutes. The algal material was then filtered out through Whatman number one filter paper and the supernatant fluid analysed for reactive phosphorus. The algal material left on the filter paper was dried at 110^{o}C and weighed. The amount of phosphorus extracted was expressed as a percentage of the dry weight of algae.

Table 8.3 shows a highly significant variation in phosphorus levels between areas. The percentage of phosphorus increases towards the Heathcote River together with the phosphorus level in the water. This agrees with the results from the literature concerning nitrogen as shown on Table 8.2. On this basis the plants at area A have normal clean water

Table 8.3 Phosphorus level in the thallus of *Ulva lactuca* as the percentage of the dry weight of the thallus.

	Sample area		
	A	B	C
	0.8	2.0	3.0
	1.1	2.0	2.9
	1.0	2.1	3.0
	1.0	2.1	3.0
Mean	0.98	2.05	2.98

L.S.D. ($P = 0.001$) = 0.26

concentrations of phosphorus while those at B and C have levels typical of eutrophic waters. To avoid complications from phosphate stored in the thallus, only plants from area A were used in the experiments.

Experimental design: Two types of experiment were designed to assess the effect of phosphorus and nitrogen concentration on growth as measured by increases in disc area.

The first method involved daily measurement of growth rate and the monitoring of the phosphorus and nitrogen levels in the culture vessels. Changes in growth rate could therefore be related to changes in the nutrient levels in the medium from the original concentration and allowed a number of different nutrient levels to be examined during this process since a decrease in concentration during the course of the experiment was expected. For this type of experiment the 3.5 l culture vessels were used. It was hoped that the large volume would reduce the effect of enclosure and give a more stable system. The main defect in this design was the limited space available under the lights which reduced the possible number

of nutrient combinations and of replications.

In the second method, smaller volumes of culture media were employed which was changed daily to maintain the initial nutrient levels and to prevent staling. This permitted a greater number of replicates and nutrient combinations. The vessels were desiccating dishes with 200 millilitres of culture medium. The discs were measured at the end of the experiment. Table 8.4 briefly summarises the aims and culture media used in the experimental programme.

Table 8.4 Summary of aims of experiments I-VI

Experiment No.	Aim Growth in response to:	Basic media
I	Phosphate additions	Provasoli ASW8 in oceanic sea water
II	Phosphate additions	Provasoli ASW8 in coastal sea water
III	Phosphate additions	Filtered and unfiltered von Stosch media
IV	Nitrate additions	von Stosch in coastal sea water
V	Nitrate and phosphate additions	von Stosch in coastal sea water
VI	Water from above sample areas	Water from sample areas without enrichment

(c) Nutrient experiments and their results

Experiment I

Aim - To determine the growth response of *Ulva* to additions of phosphate to the medium.

Methods and experimental conditions: The temperature

of the culture vessels was kept at 17°C with a photoperiod of 16 hours light and 8 hours dark.

Four 1 centimetre discs were placed in separate compartments of twelve 3.5 l culture vessels as previously described. ASW8 medium, made up in water collected 30 miles off the Canterbury coast, was added to eleven of these vessels while the twelfth was filled with the same medium made up in water collected from the mouth of the Estuary at high tide.

The nitrate level of all vessels was built up to 0.6 gram per cubic metre by additions of $NaNO_3$ while the phosphate level was varied by additions of K_2HPO_4 to give the following series:

0.02^{+}, 0.04^{++}, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 $g/m^3 P$

+0.02 was the concentration of the oceanic stock

++0.04 was the concentration of the coastal sea water stock.

Results of Experiment I.

(A) Growth response to phosphorus additions: Fig. 8.2 gives the percentage increase in disc area against the reactive phosphorus level. At day one there was no significant growth below 0.4 gram per cubic metre P. Above 0.4 gram per cubic metre the growth tended to increase with increasing phosphorus but these differences are not statistically significant at the 99% confidence level. At day two, growth was significantly less in levels below 0.3 gram per cubic metre but with no difference between levels above 0.3 gram per cubic metre. By day three the discs in concentrations above 0.7 gram per cubic metre produced zoospores which invalidates these results. From day three to day five there was an approximately linear

response to increasing phosphorus concentrations between 0.2 and 0.5 with the maximum increase at 0.5 gram per cubic metre.

The algae in the tank containing the coastal water had a higher growth rate than would be predicted from the phosphorus concentration.

(B) Growth rate against time: The relative growth rate curves as shown on Fig. 8.3 all have a similar shape but the higher phosphorus levels have a steeper slope. The growth rate was highest between days three and four. Unfortunately, the cooling unit did not function consistently during this experiment, resulting in the temperature fluctuations as shown on Table 8.5.

Table 8.5 Temperature in ^{o}C

	Day 1	Day 2	Day 3	Day 4	Day 5
Range	16.5-17.5	16.5-17.5	18-20	13-15	16.5-17.5
Mean	17	17	19	14	17

The high rate of growth between day three and day four is probably the result of the higher temperature during this period.

Results of phosphorus analysis

Table 8.6 Reactive phosphorus levels in g/m^3

Concentration at Day								
0	0.02	0.04	0.1	0.2	0.3	0.4	0.5	0.6
3	0.01	0.02	0.05	0.15	0.3	0.35	0.44	0.55
0	0.7	0.8	0.9	1.0				
3	0.65	0.75	0.81	1.1				

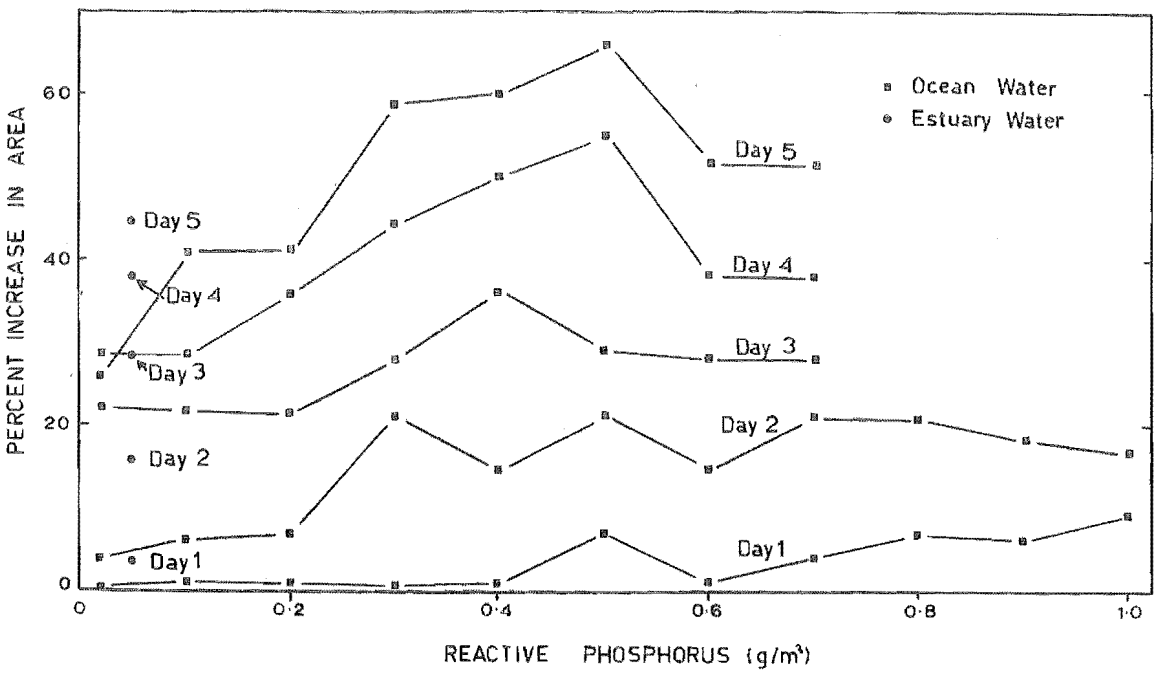


Fig 8·2 Growth rate against phosphorus concentration, experiment I

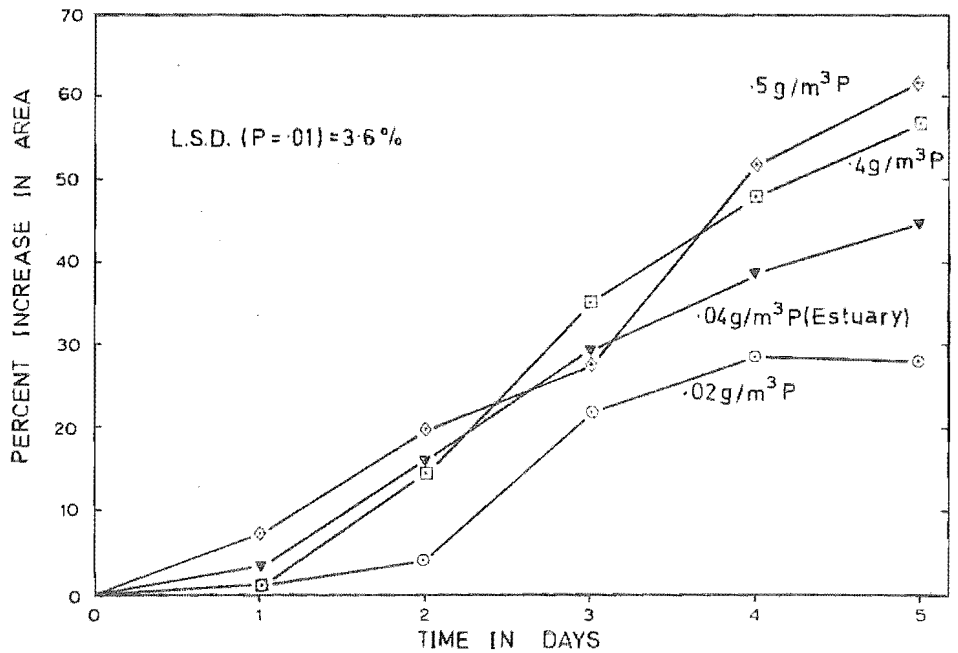


Fig 8·3 Growth rate against time at different phosphorus levels, experiment I

The levels at day three were generally lower than the initial concentration with the exception of the highest concentration.

Discussion of results for Experiment I: The maximum rate of growth occurred at 0.5 gram per cubic metre P with slightly lower growth rates at higher concentrations and decreasing rates of growth at lower levels. This trend is most marked in the later stages of the experiment. The main changes during the experiment were the increases in growth at the lower concentrations and marked increases in the tank with an initial level of 0.5 gram per cubic metre. Increases in growth are the reverse of what would be expected from the changes in phosphorus levels which decreased during the experiment. The rate of growth could therefore be expected also to decrease with time, especially in the lower concentrations. Growth, therefore, appears to be related to the initial concentration rather than the levels pertaining in the latter stages of the experiment.

The decrease in phosphate concentration represents a much greater loss of phosphate than can be attributed to algal uptake. For example, a decrease of 0.05 gram per cubic metre requires a loss of 0.17 milligram of P which is greater than the dry weight of the Ulva discs in each tank. This aspect will be studied in greater detail in the following experiment.

The coastal water gave consistently better growth than the oceanic water, possibly due to the presence of one or more growth factors or micronutrients. As the coastal water is also more relevent to the Estuary, it was used in all of the subsequent experiments.

Experiment II

Aim - The general aim was the same as that of experiment I, namely the investigation of the growth response to additions of phosphate but by using smaller increments of phosphate, the responses to concentrations below 0.5 gram per cubic metre P were examined in greater detail. The following series was used:

0.04, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5 g/m^3

The concentrations of reactive phosphate and nitrate in the culture vessels were also tested on day two and day three in order to study more closely the decreases in these elements which were indicated in experiment I for phosphorus.

Only coastal sea water was used and the temperature was maintained between 16.5°C and 17°C. The photoperiod was altered to 14/10 as this is more natural and relevant to the Estuary and is recommended by Kornman (1972).

Results: The percentage increase in growth is plotted against the phosphate concentration in Fig. 8.4. Zoospore production occurred in the 0.2 gram per cubic metre P level at day two and in the 0.5 gram per cubic metre P concentration at day five. Very little growth occurred in levels below 0.25 gram per cubic metre and the discs in these levels began to disintegrate after day three. They were, therefore, excluded from the results along with the reproductive discs. The most rapid and consistent growth occurred at 0.45 gram per cubic metre with slower growth at levels between 0.25 and 0.45. The rates of increase with time were basically linear as in experiment I (Fig. 8.5).

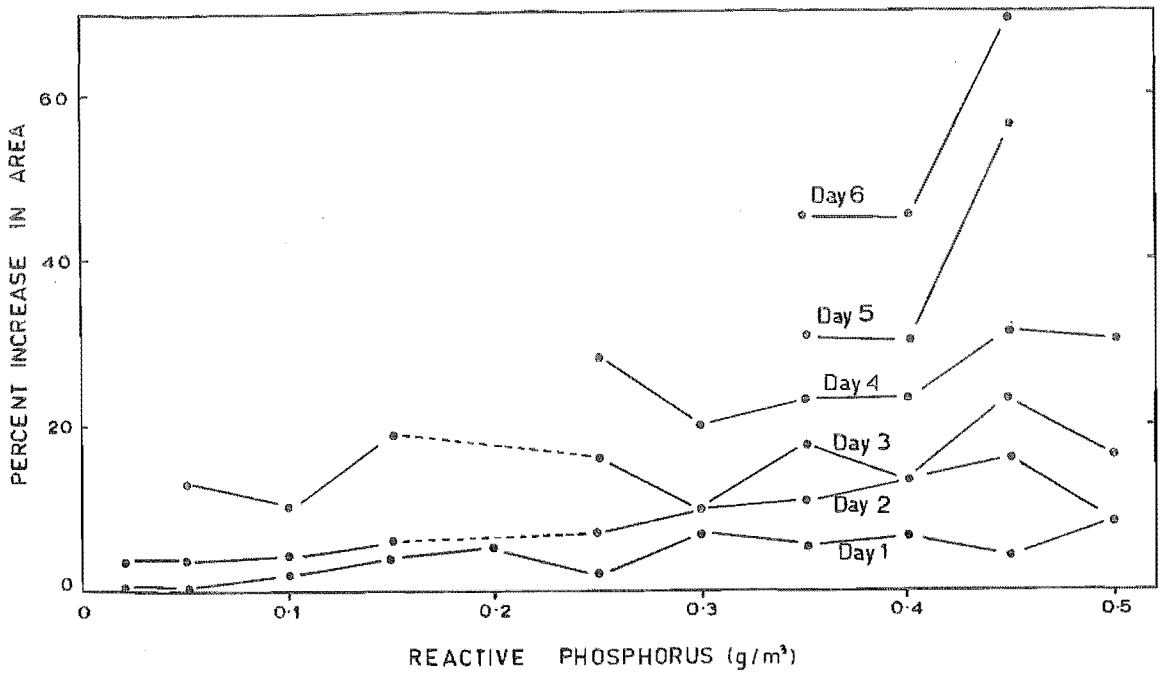


Fig 8·4 Growth rate against phosphorus concentration, experiment II

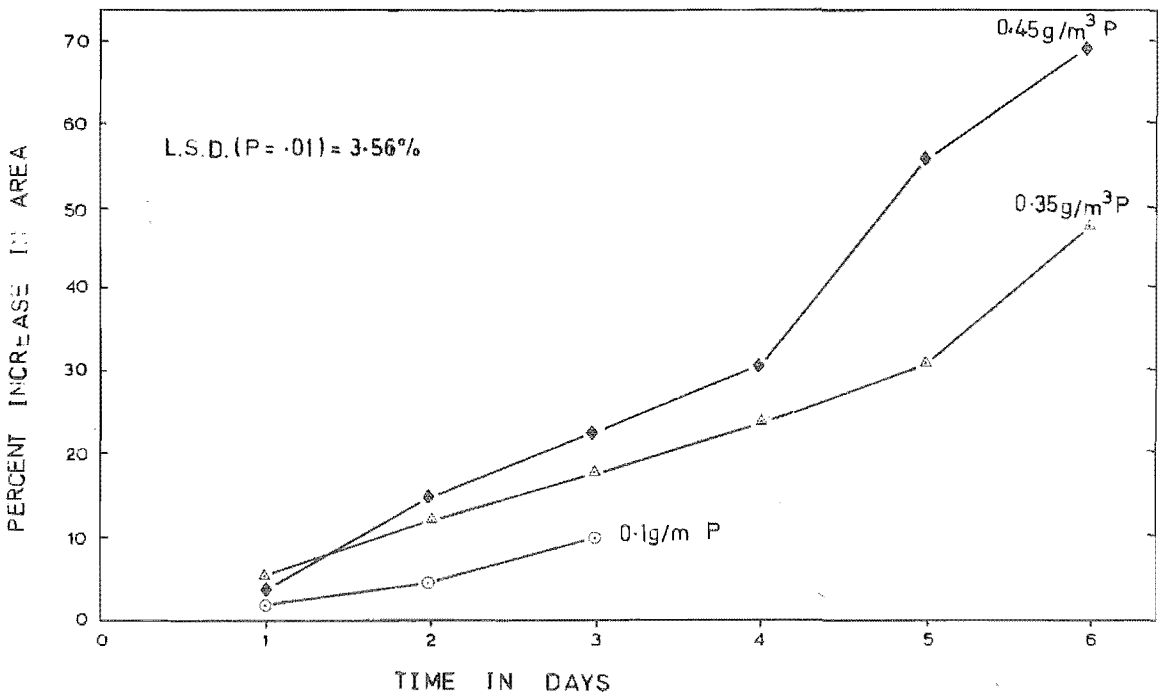


Fig 8·5 Growth rate against time in different phosphorus levels, experiment II

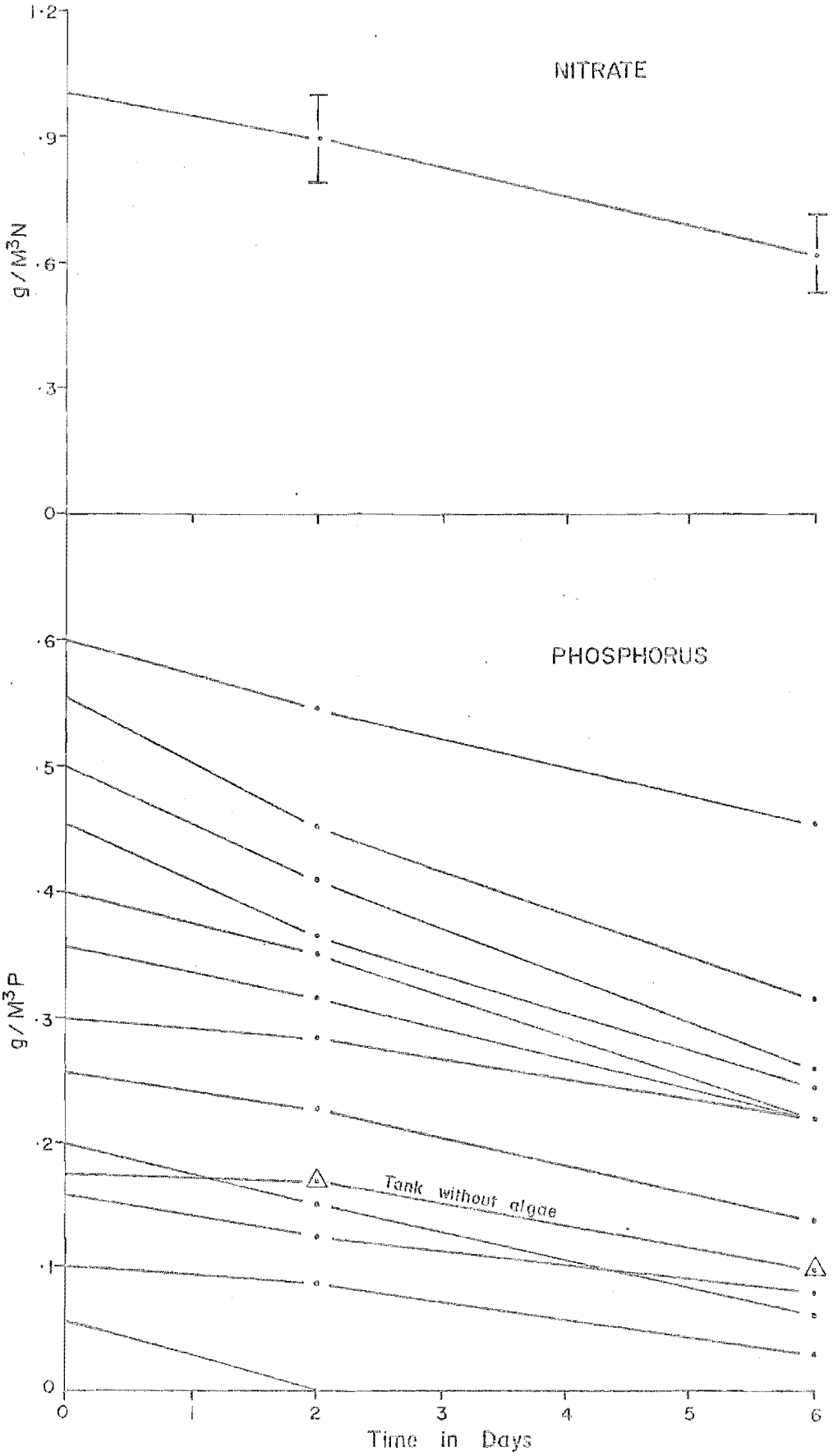


Fig. 8·6. Changes in reactive phosphorus and nitrate levels during experiment II.

Changes in nutrient levels during the experiment: As shown on Fig. 8.6, the initial nitrate nitrogen levels were between 0.9 and 1.1. The levels decreased to 0.9 ± 0.1 on day two and to 0.55 ± 0.1 on day six. There was no significant difference between the tanks. It appears that these concentrations are above the range for which the Brucine method as used by the Zoology Department is reliable. The rate of loss increased with time. Phosphorus followed the same pattern with all levels showing significant decreases in concentration. The control tank without algae also decreased in both nitrate and phosphorus concentration at a similar rate to those with algae. There had been very little change in the phosphorus level at day two but a significant decrease occurred between day two and day six.

Discussion of results from experiment II: The results are basically similar to those of experiment I. Growth rates were similar and increased with increasing phosphorus levels up to 0.45 gram per cubic metre.

The decrease in phosphorus levels in the medium noted in experiment I was confirmed and a decrease was found to occur also in the nitrate N levels. The amount of decrease in both nutrients precludes algal uptake as the main cause. This is supported by a similar course of events in the tank lacking algae. There are a number of possible explanations for this phenomenon. Firstly, there is adsorption of the phosphate molecules on to the sides of the tanks. The amount adsorbed would reflect the concentration in the water which fits the observed results. The rate of adsorption would become smaller with time as available sites were utilised, but the results on the contrary generally indicate an increasing rate of loss with time.

A second possibility is uptake by bacteria. Although the sea water was autoclaved, contamination could occur from the air and on the discs of algae and the number of bacteria would therefore tend to increase during the experiment. This may explain the increasing rate of loss of P and N from the solution.

It is also possible that the P and N were changed to a form which was not detectable by the analytical methods used. The nitrate, for example, may have been converted to nitrite or ammonium. This type of conversion may also increase as the bacteria multiplied.

Although aseptic cultures can be used, this leads to a number of technical difficulties and has not produced successful results in studies of other workers. Provasoli (1957) failed to achieve normal growth of Ulva lactuca under these conditions. Since lack of bacteria is unnatural, extrapolation from in vitro to in vivo results is more difficult under those circumstances. Also the addition of antibiotics to culture media has been shown to affect the chloroplasts of some algae (Provasoli, 1948; Steffensen, 1974).

Experiment III

Aim - To investigate the effect of filtering out the particulate matter from the sea water stock on the growth rate of Ulva and on the nutrient concentration.

Method: Two series of phosphorus levels of 0.02, 0.2, 0.4, 0.6, 0.8 and 1.0 gram per cubic metre were made, one based on estuarine water filtered through Whatman number one filter paper, the other based on water which was not filtered. The metal and vitamin mix in the ASW8 medium was replaced by

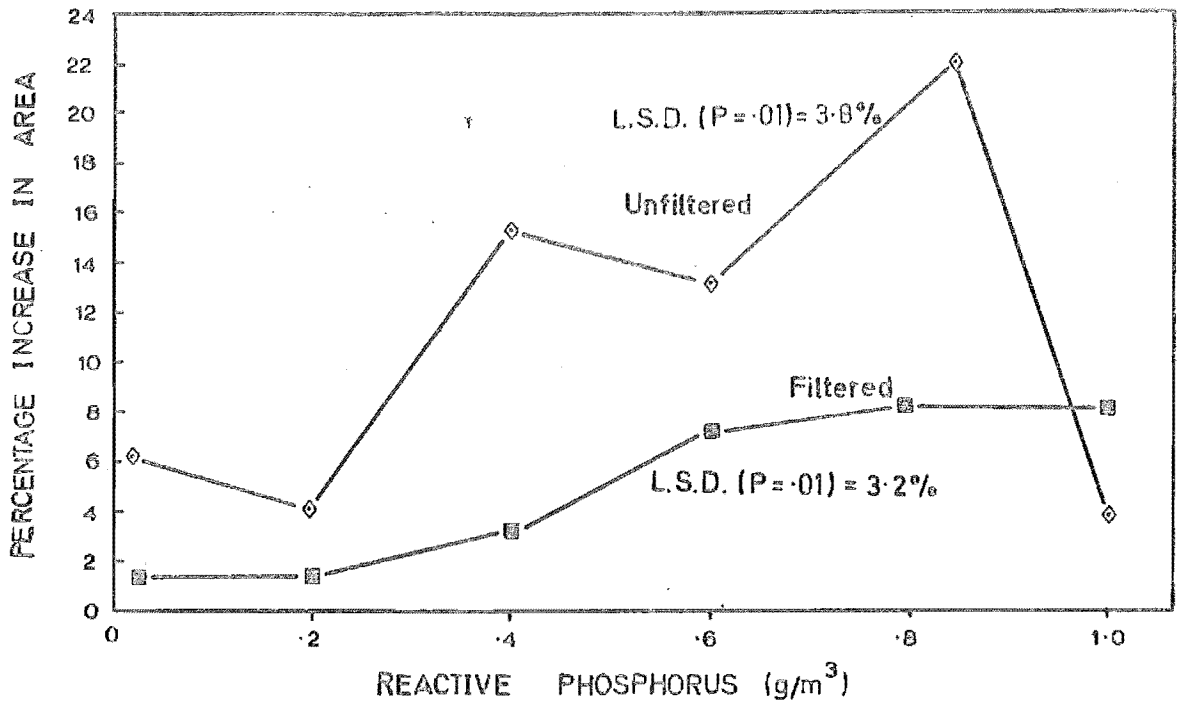


Fig 8·7 Growth of Ulva Lactuca in filtered and unfiltered water; experiment III

the simpler mix as recommended by von Stosch (1957).

All other conditions were the same as in experiment II.

Results: As shown on Fig. 8.7, the growth was higher in the unfiltered water than in the filtered water. The response in relation to the phosphate concentration in the unfiltered water was more variable than that in the filtered water which was similar to previous experiments.

As shown in Table 8.7, the reactive phosphorus decreased more in filtered water than in the unfiltered water.

Table 8.7 Changes in reactive phosphorus levels in $g/m^3 P$

Initiation		0.02	0.2	0.4	0.6	0.8	1.0
Day 3	Filtered	0.01	0.01	0.25	0.44	0.62	0.9
	Unfiltered	0.01	0.05	0.3	0.56	0.66	0.9

L.S.D. (P.01) = 0.02 g/m^3

Discussion of results: The presence of particulate matter in the unfiltered medium maintained a higher reactive phosphorus level which may explain the higher growth rate. It is possible that there is an equilibrium between the dissolved or reactive phosphorus and the phosphorus bound to the particles. As the reactive phosphorus decreased, more of the bound phosphorus would go into solution.

There may also be similar equilibria for other nutrients such as nitrogen and organic growth factors. These factors may also promote better growth in the unfiltered water.

Although unfiltered water is a more natural medium and gave better growth, the results are more variable and more difficult to interpret. As it is easier to identify the cause of any growth changes in filtered water, it was retained for subsequent experiments.

Experiment IV

Aim - To investigate the growth responses of Ulva lactuca to additions of nitrate at the optimal phosphate level.

Method: The conditions and media were the same as in previous experiments except that phosphorus was kept at 0.6 gram per cubic metre P while $NaNO_3$ was added to give the following series:

0.02, 0.2, 0.4, 0.6, 0.8 $g/m^3 N$.

Results: The growth at 0.02 gram per cubic metre N increased by 10% which was significantly lower ($P = 0.01$) than the 20-24% increase found for all other levels.

Discussion of Results: This uniformity of response was a rather puzzling result as nitrogen is generally regarded as an important nutrient. It is possible that nitrogen is not limiting at 0.2 gram per cubic metre and above but limiting at 0.02 gram per cubic metre N. The second possibility is an interaction between phosphorus and nitrogen in the way they affect growth. This hypothesis is examined in experiment V.

Experiment V

Aim - To investigate the effects of various combinations of reactive phosphorus and nitrate concentrations on the growth of Ulva lactuca.

Method: The temperature, lighting and basic culture medium was the same as that used in experiment IV but the nitrate and phosphorus levels were varied in the following manner. Nitrate concentrations of 0.03, 0.3, 0.6, 0.9 and 1.2 gram per cubic metre N were combined with phosphorus concentrations of 0.01, 0.3, 0.6, 0.9 and 1.2 gram per cubic metre P in a 5 x 5 factorial arrangement to give 25 combinations of N and P. The lowest levels were those of the basic medium. There were four replicates of each combination with one disc of Ulva per replicate. The culture medium was renewed daily and the discs were measured after five days.

Results: As the results are in percentages, an angular transformation using tables in Rolf and Sokal (1969) was performed in order to meet the requirements for an analysis of variance, the results of which are shown in Table 8.8.

Table 8.8 Analysis of variance

Source of variation	Degrees of freedom	Mean square	Variance ratio
Nitrogen main effects	4	400.64	57.4***
Phosphorus main effects	4	154.74	22.2***
Interaction N x P	16	32.08	4.6***
Error S.	72(1)		

*** P 0.001.

(1) Three values were estimated.

Table 8.9 Least significant differences (L.S.D.)

Significance levels	N, P main effects	P at each level of N N at each level of P
5%	1.67	3.72
1%	2.21	4.95

As shown in Table 8.8, there is a highly significant interaction between N and P in their effect on growth. The L.S.D. for P at each level of N and N at each level of P are more meaningful. The results as the means of the transformed data are shown in Table 8.10.

Table 8.10 Results of experiment V as transformed means

Phosphorus concentration	Nitrogen concentration in g/m^3 0.03	0.3	0.6	0.9	1.2	Mean
0.01	17.3	19	26	19.8	16.8	17.8
0.3	24.0	25.4	28.3	23.2	21.6	24.9
0.6	31.4	30.8	30.6	21.4	19.1	26.6
0.9	30.2	30.7	31.6	21.3	18.8	26.6
1.2	28.8	29.4	29.9	18.1	17.2	24.7
Mean	26.4	27.04	29.25	20.7	18.7	

It is clear from Table 8.10 that the 0.9 and 1.2 gram per cubic metre N levels have an inhibiting effect at all phosphorus levels. Unfortunately, it is not possible to allow for this by partitioning the degrees of freedom as this is an apriori method not included in the original design of the experiment.

The trends in the data are more clearly shown in graphs of the retransformed means. Figs 8.8 and 8.9 are two dimension graphs while Fig. 8.10 gives the results as a response surface with the results from the previous experiment added to allow the curves to be rounded off.

According to Fig. 8.9, the main effect of nitrate additions is the stimulation of growth at levels up to 0.6 gram per cubic metre N followed by decreasing growth in the 0.9 and 1.2 gram per cubic metre N concentrations. The response to nitrogen is also affected by the phosphorus concentrations.

The effect of phosphate is to increase growth up to 0.6 gram per cubic metre P. The increase from 0.01 to 0.3 gram per cubic metre P has the effect of increasing the growth reponse at all N concentrations. At 0.6 and 0.9 gram per cubic metre P the maximum growth rate is reached at the lowest N concentration and maintained until growth decreases in the high N concentrations. This inhibition at the high N becomes greater as the P concentration increases from 0.3 to 1.2 gram per cubic metre. The 1.2 gram per cubic metre P curve follows the 0.9 and 0.6 P curves but at a slightly lower level. This difference is not statistically significant at the 95% confidence level.

The interactions between P and N are more easily seen

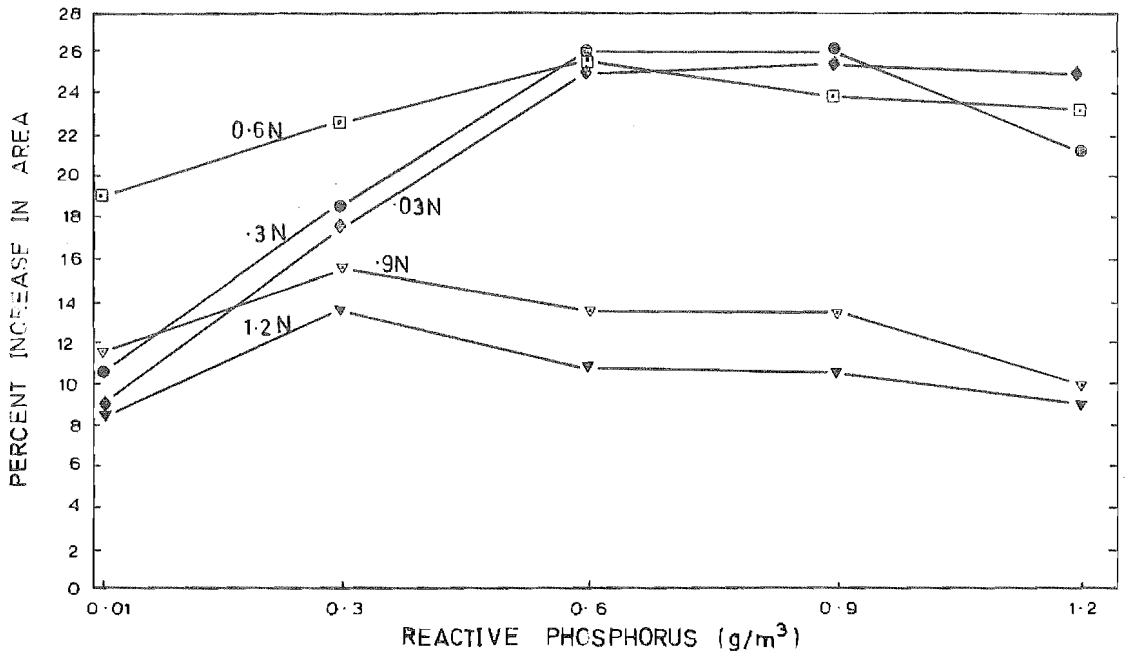


Fig 8·8 Growth response to phosphorus at different levels of nitrate, experiment V

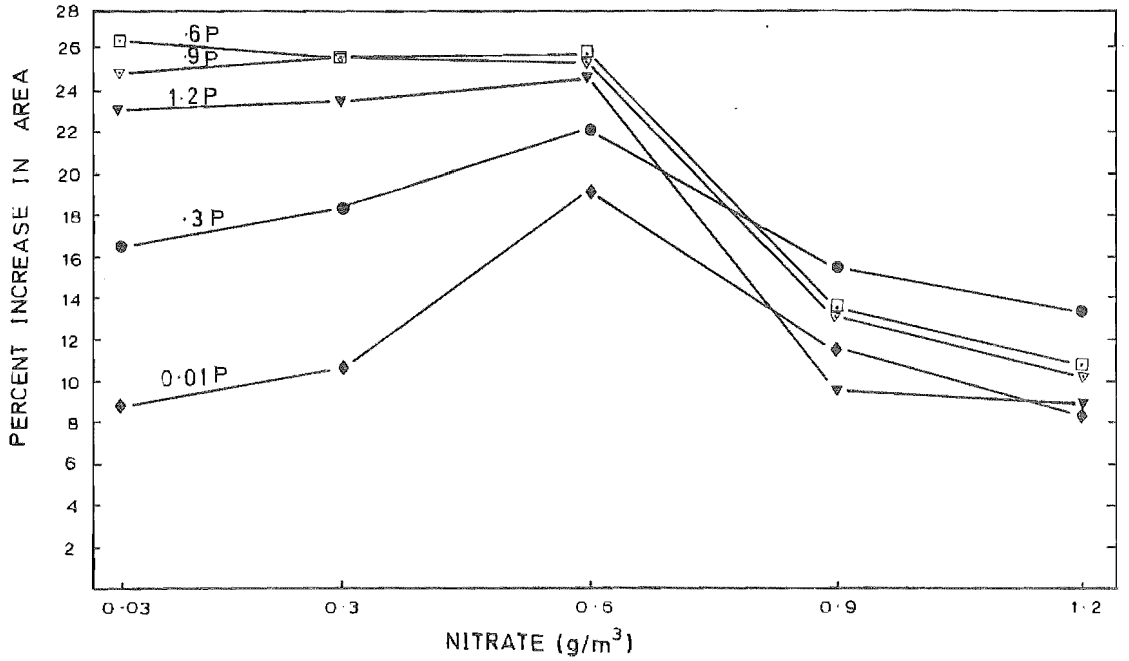


Fig 8·9 Growth response to nitrate additions in varying levels of phosphorus, experiment V.

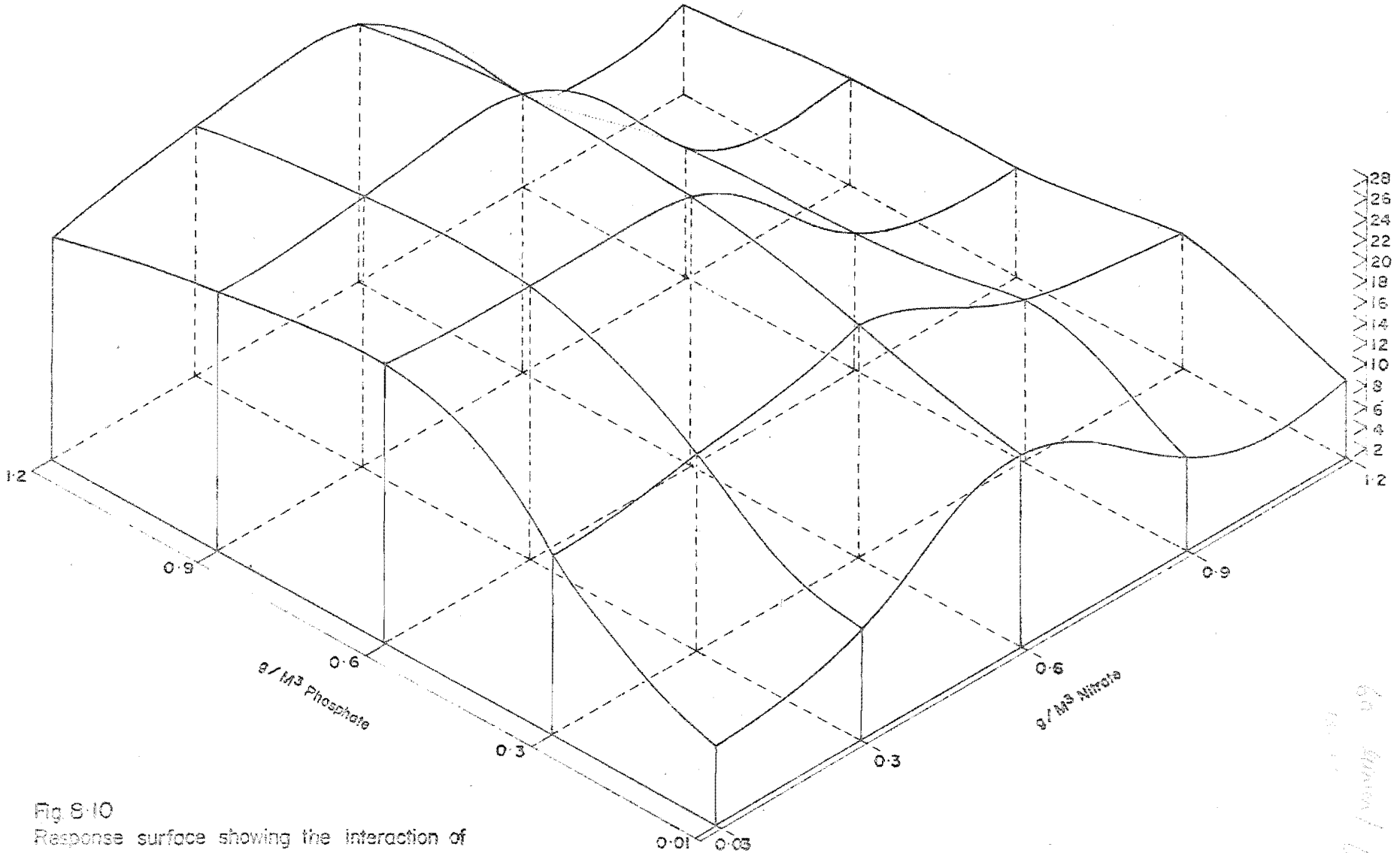


Fig. 8·10
Response surface showing the interaction of phosphate and nitrate on growth of Ulva lactuca.

on the response surface Fig. 8.10. Both elements are below their optimum in the square bounded by the 0.6 P and 0.6 N levels. Within this area the response to either element is affected by the concentration of the other. For example, the point representing growth at (0.3 P, 0.3 N) is higher than either the (0.3 P, 0.03 N) or the (0.01 P, 0.3 N). At P and N concentrations above the optima the response surface is dominated by the growth response stimulated by high P and the inhibitory effect of high N concentrations. The plateau of response at 0.6 gram per cubic metre P and above and 0.6 gram per cubic metre N and below results from stimulation by P, while the valley formed at the 0.9 and 1.2 gram per cubic metre N results from the inhibitory effect of high N concentrations. This inhibitory effect of high N is moderated at the 0.3 P concentration but intensified at 1.2 P concentration. This supports the suggestion that the 1.2 P concentration may be supra-optimal. In the combination with the lowest P concentration and the highest N concentration growth may be limited by both a lack of P and an excess of N.

Discussion and summary of experiments I to V:

Experiment V gave the most useful results as it brought out the way in which phosphate and nitrate levels interact in their effect on growth of Ulva lactuca. These results also explain the uniform response to nitrate additions in experiment IV, as the phosphate level was sufficient to give optimum growth irrespective of the nitrate levels used. The high levels of nitrogen used in experiments I, II and III also explain the relatively small differences in response to phosphate additions and the relatively large response in the lower phosphate levels. If these phosphate-nitrate

interactions had been tested earlier in the programme, the detailed examination of the independent effects of these elements would have been more profitable.

The results of earlier experiments on the effect of phosphate additions confirm the results of experiment V: there is an approximately linear response to added phosphate up to 0.6 gram per cubic metre with very little effect from further additions. Experiment II indicated that maximum growth may occur at concentrations down to 0.45 gram per cubic metre.

The response to nitrate additions is not described in the same detail as to phosphate due to the over-riding effect of the phosphate level in experiment IV.

The smaller volume of culture medium in experiment V gave more reliable results than the larger volumes of the previous experiments as it permitted a greater number of nutrient combinations to be studied and as it made possible more replicates for each nutrient combination. Besides, with smaller volumes, the medium could be renewed more frequently, maintaining a more stable nutrient concentration and preventing excessive increases in bacterial numbers. The additional time involved in changing solutions, however, precluded daily measurement of growth rates.

The results in this study closely resemble those obtained by Waite and Mitchell (1972b) for Ulva lactuca in spite of the different techniques employed both for growth measurement and for the nitrogen source. The above authors measured growth as net productivity from C^{14} uptake and added nitrogen as ammonia. This similarity of results gives mutual support to the two growth measurement techniques and indicates

that addition of nitrogen as ammonia and as nitrate has the same effect on growth. This differs from the results by Andersson (1942) who found nitrate less toxic than ammonia.

As a greater number of P/N combinations was used in this study, the response surface as shown in Fig. 8.10 is defined in more detail than that described by Waite and Mitchell but the general trends are the same. That is, the optimal growth occurred at similar levels, inhibition was recorded in high nitrogen concentrations but not in high phosphorus levels and interaction between phosphorus and nitrogen occurred below the optimal levels.

Experiment VI

Aim - To investigate the growth of Ulva lactuca in water collected from within the Estuary.

Method: Water samples were collected from above the sample areas at high tide and used as the culture medium without additions of any kind. The reactive phosphorus and nitrate concentrations were determined for each sample and the media changed daily. There were five replicates for each water sample with each containing one disc which was measured after five days.

Results:

(A) Growth: The increase in disc size varied from 8% to 10% (mean 9.3) but there were no significant differences between discs in different water samples.

(B) Nitrate levels: The nitrate levels varied between 0.08 and 0.12 gram per cubic metre N but any differences between samples were not statistically significant. This appeared to be the result of analytical errors rather than of

variability within the water samples. As described in the field methods, the analysis was carried out by members of the Zoology Department using the Brucine method. This technique, although widely used, is not easily applied and the variation in the results may reflect the analyst's inexperience with this difficult technique. Within the time available it was not possible for the author to personally carry out the analyses and nitrate specific electrodes, which would have overcome this problem, did not arrive in time for use in this study.

(C) Reactive phosphorus: The phosphorus concentrations are listed below in ascending order as grams per cubic metre P.

Table 8.11 Phosphorus concentrations used in experiment VI

A	(1)	F	B	E	C	D
0.0	0.02	0.026	0.035	0.08	0.09	0.095

L.S.D. (P = 0.01) = 0.013
(1) = sample from Monck's Bay

Area A has significantly lower reactive phosphorus than the other areas with the exception of quadrat (1). Areas E, C and D were all significantly higher than the rest and area F and area B had intermediate values.

Discussion of results of experiment VI: The concentrations of nitrate and reactive phosphate are at the lower end of the series used in the previous experiments which may explain the uniformity of the growth response in spite of the differences in phosphorus levels. The level of growth is very similar to that obtained in equivalent

concentrations of previous experiments in which the media were enriched with various metals and vitamins. This suggests that at least for this level of growth the micronutrients added in the enrichments were already adequately supplied by the coastal sea water stock.

2) THE EFFECT OF TEMPERATURE ON GROWTH OF ULVA LACTUCA UNDER LABORATORY CONDITIONS

Materials and Method

The culture medium, vessels and growth criteria were the same as those used in experiment V but the vessels were kept in a range of temperatures. The temperature gradient was created by cooling one end of an aluminium plate and heating the other. The plate was 1.05 metres long, 48 centimetres wide and 6 millimetres thick. One end was cooled in a refrigerated water bath containing a mixture of water and ethylene glycol, the opposite end was heated thermostatically by controlled heating elements fixed to upper and lower sides of the bar. By varying the temperature at the heated end, different temperature gradients could be created along the plate. Polystyrene was used to reduce heat loss from the underside of the bar. Lighting was provided by six cool white fluorescent tubes which gave a light intensity of 60 lumen per square foot. The temperature gradient was stable throughout the experiments but the isotherms on the bar tended to curve towards the edge.

The culture vessels were placed in rows of four at regular intervals along the bar. The outer margins of the bar were not used so as to avoid the curved portion of the isotherms and the small reduction of light intensity in this

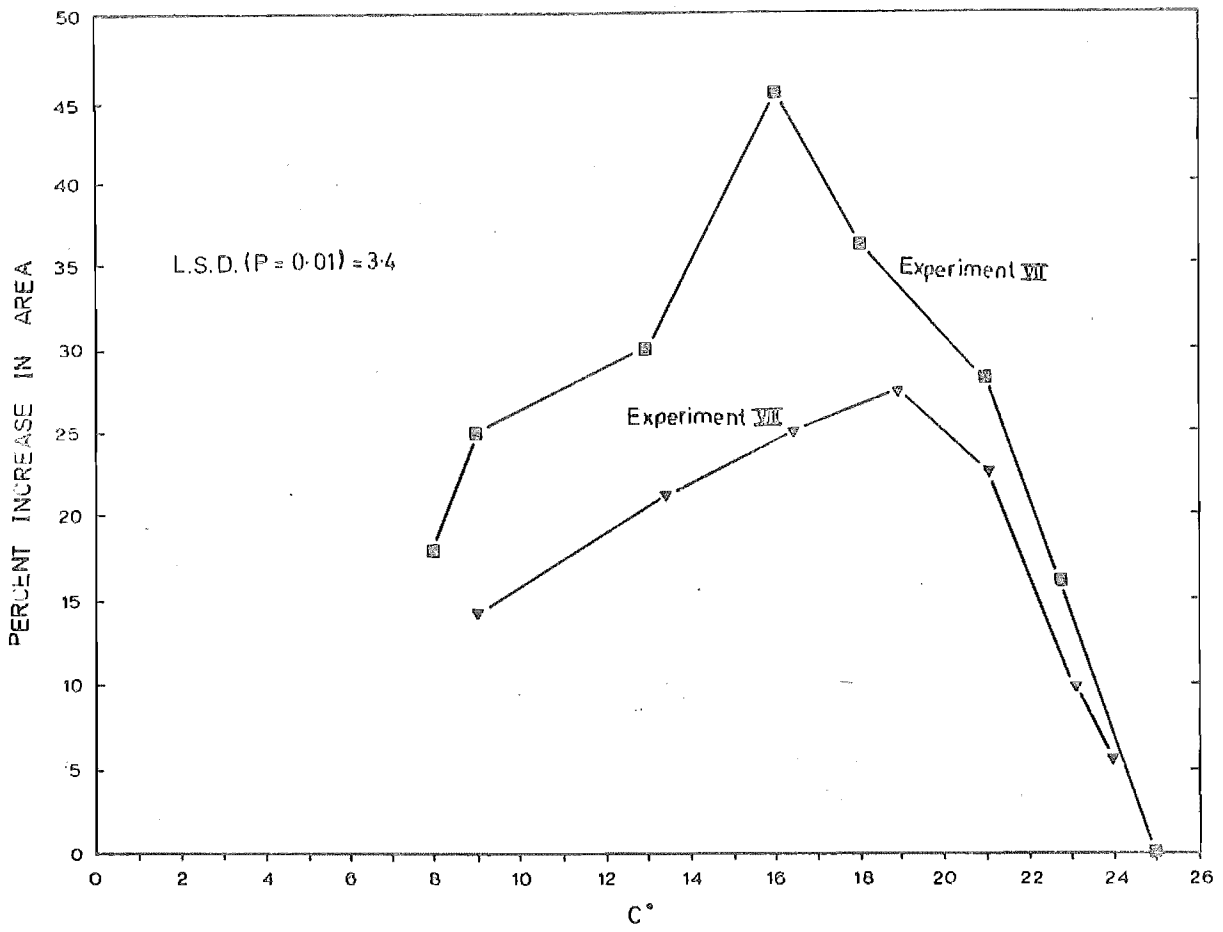


Fig 8·11 Growth response at different temperatures of discs from attached plants (Expt VII) and detached plants (Expt VIII)

area. To eliminate condensation in the vessels at temperatures higher than ambient, the vessels were covered with a sheet of plate glass heated by a modified automobile demisting system. The temperature of the culture medium in each tank was checked four times daily and found to be constant within each row. The light intensities were also checked and found to be constant for all vessels. Three discs were used in each vessel. In experiment VIII the discs were cut from drift plants from McCormack's Bay. The discs were measured after seven days.

Results of experiments VII and VIII: Fig. 8.11 shows the percentage increase in growth against temperature. The

growth increases very sharply to an optimum at 16°C for the discs from attached plants and to an optimum at 19°C for the discs from drift plants. In both cases, growth is inhibited at levels higher than 20°C and ceases completely at 25°C.

The attached plants gave a greater growth response than the drift plants from McCormack's Bay but reached the maximum increase at a lower temperature. As the plants from McCormack's Bay were larger, and presumably older, than the attached plants, the smaller response may be due to reduced metabolic activity with age. The higher optimum may be a physiological adaptation to the higher summer temperatures in McCormack's Bay.

Effect of light intensity and photoperiod on Ulva and Enteromorpha

This aspect was not studied by the author but was examined by MacKenzie (1973) and Green (1973) using sporelings of Ulva lactuca and Enteromorpha ramulosa plants collected from the Avon-Heathcote Estuary. The growth of sporelings in the first week after germination was measured under 16/8 and 8/16 photoperiods and at 26, 22, 17 and 10 lumen per square centimetre. For both species, growth increased with light intensity but the greatest contrast was between the consistently high growth rate at all light intensities in the 16 light and 8 dark photoperiod and the lower growth rate in the 8/16 photoperiod.

Waite et al (1973) found that increases in light intensity from 480 to 1000 ft candles did not alter the growth rate significantly in Ulva lactuca which agrees with the results from this study.

CHAPTER IX

THE RELEVANCE OF THE LABORATORY RESULTS TO GROWTH IN THE FIELD

There are varying opinions as to the relevance of results from artificial laboratory conditions to the processes in the natural environment. Provasoli (1958) supports the use of the experimental approach on the basis that stresses caused by extreme laboratory conditions are no greater than those of the "continuous challenge of nature to the potentialities of the organism". Provasoli and Pintner (1956) also note a "remarkable parallelism between *in vitro* results and the composition of natural waters", with the exception of phosphorus. Chu (1942) and Rodhe (1948) found that 10 to 100 times the natural levels of phosphorus were required to produce bloom densities of plankton in culture.

The results from the present study support Provasoli's confidence in laboratory experiments and in contrast to the observations by Chu and Rodhe indicate that *in vitro* results for phosphorus also parallel growth in natural waters.

1) GROWTH RESPONSE TO PHOSPHORUS

The phosphorus concentrations used in the experiments ranged from that of the sea water entering the Estuary to levels of about one third of that in the sewage effluent. The determination of mean concentrations for each sample area

or for a particular time of the year is complicated by the tidal influence as discussed in Chapter VII, but it is clear that phosphorus levels increase from the mouth of the Estuary to the western region due to the discharge of sewage effluent. The mean concentrations for each sample area, as shown on Fig. 6.26, are all well below the *in vitro* optimum and, as shown by experiment VI, would result in relatively slow growth, that is approximately 10% increase in five days. The upper limit of the concentration ranges, however, reach values which stimulated rapid growth rates in the laboratory with the optimum of 0.5 to 0.6 grams per cubic metre being exceeded at area D. As mentioned in Chapter VIII, it is likely that the concentration in the superficial water surrounding the algae at low tide may be higher than the ranges given in Fig. 6.26. It is possible that the algae may be able to assimilate excess phosphorus at or near low tide and utilise the stored phosphate for growth at high tide when the levels in the water are low. The high phosphorus levels in thalli from area C support this hypothesis and suggest that at least for part of the time the phosphorus concentrations in this area were in excess of the algae's requirements. This suggests that the maximum values for phosphorus may have more ecological significance than the mean or high tide values which are largely the result of the very low concentrations in the incoming sea water. On this basis areas C and E have approximately twice the phosphorus concentration found at areas A, B and F with area D having up to four times these levels. These differences should produce significant differences in rates of growth. The very high standing crop found at areas C and D supports this but may also be the

result of algae drifting in and of the lack of herbivors. The relatively low standing crop at area E, in spite of its high phosphorus concentration, is satisfactorily explained by the long exposure period and the lack of attachment sites.

The above discussion refers to reactive phosphorus as determined from filtered water which may underestimate the amount of phosphorus available to the algae. The high rate of growth in the unfiltered water as shown in experiment III indicates that some of the phosphorus bound to particulate matter in the water may also be available to the algae. The unfiltered water also had a more stable reactive phosphorus level which suggests that the particulate matter acts as a phosphate buffer.

2) GROWTH RESPONSE TO NITROGEN

As mentioned in Chapter VIII, the nitrate values from the Estuary do not represent the total nitrogen available to the algae but they should indicate the relative nitrogen status of the areas. The main discrepancy will occur at area D which may be subject to high ammonia levels due to its proximity to the sewage effluent.

The mean values of nitrate, in spite of being approximately twice those of reactive phosphorus, are still within the range that gave only slow growth in the laboratory. The upper limit of the ranges, however, reach the optimum at areas C, D, E and F. The very high nitrogen levels in the thallus of the algae as shown in Table 8.1 support the contention that over much of the Estuary more nitrogen is available to the algae than they require for growth.

The nitrogen concentration in the sewage effluent is approximately 30 fold greater than that found to inhibit growth in the laboratory. The areas immediately adjacent to the effluent discharge points may have toxic levels of nitrogen, which offers an explanation for the complete lack of algae there as shown by Figs 6.1 to 6.10.

Both phosphorus and nitrogen levels in the Estuary are below the *in vitro* optimum over most of the period during which the algae are covered by the tide and probably never reach optimum concentrations near the mouth of the Estuary. Of the two nutrients, phosphorus is always further below its optimum than is nitrogen and, as additions of phosphorus had a greater effect on growth in the laboratory, it appears to be the main limiting factor on the Estuary. If the volume of discharged sewage effluent increases as predicted in Table 1.2, the concentrations of phosphorus and nitrogen will also increase. The levels which stimulate rapid growth will therefore occur over a wider area of the Estuary and for a longer period of time for each tidal cycle. This will increase the potential rate of growth, resulting in increased algal standing crop where other conditions such as temperature and exposure are also favourable for growth.

3) GROWTH RESPONSE TO TEMPERATURE AND LIGHT CONDITIONS

In the field, light intensity, photoperiod and temperature all increase together and all three may influence the seasonal variation in productivity. The experiments by Mackenzie and Green indicate that light intensity and photoperiod may be important but the experimental design used

in this case did not preclude temperature increase in the higher light intensities and longer photoperiod. On the other hand, response to temperature is more clearly defined.

The experimental temperatures used in the laboratory range from the levels typical of winter or early spring over the spring and summer levels to values higher than those recorded for the Estuary. There is very good agreement between the temperatures which produced the maximum stimulation of growth in the laboratory and the temperatures at which maximum growth occurred in the field. This gives good support to the hypothesis that temperature is the main factor in determining the seasonal growth pattern. The correlation between unusually high standing crop and above average summer temperature also supports this theory.

4) THE RELEVANCE OF A LIMITING FACTOR EQUATION TO THE RESULTS OF THIS STUDY

The field and laboratory results indicate that the productivity and standing crop levels are influenced by a number of interdependent variables whose effects do not fit into a limiting factor concept. Waite and Mitchell (1972b) suggested that the nutrition of Ulva lactuca was best described by a limiting factor equation as formulated by Baule (1918) and modified by Verduin (1964). In the following section the application of Verduin's equation to the results of this study will be discussed.

Verduin's equation

$$Y = Y_{opt.} \, (1-2^{-x}) \, (1-2^{-y}) \, (1-2^{-z}) \text{ etc.}$$

Y = Photosynthetic yield per m^2

Y opt. = Yield obtainable if all factors are optimal

x, y etc. = Factors limiting photosynthesis

All factors are expressed in terms of their effect on Y opt. by assuming that x equals unity when y is equal to $\frac{1}{2}$Y opt. This relationship is shown graphically in Fig. 9.1 for the response curve as used by Verduin (1964). For curves of this type it is evident that increasing values of x result in approximately linear increases at low levels of x, but at higher values the effect on yield becomes progressively smaller, while the yield curve approaches a value of 1 (Y opt.) asymptotically.

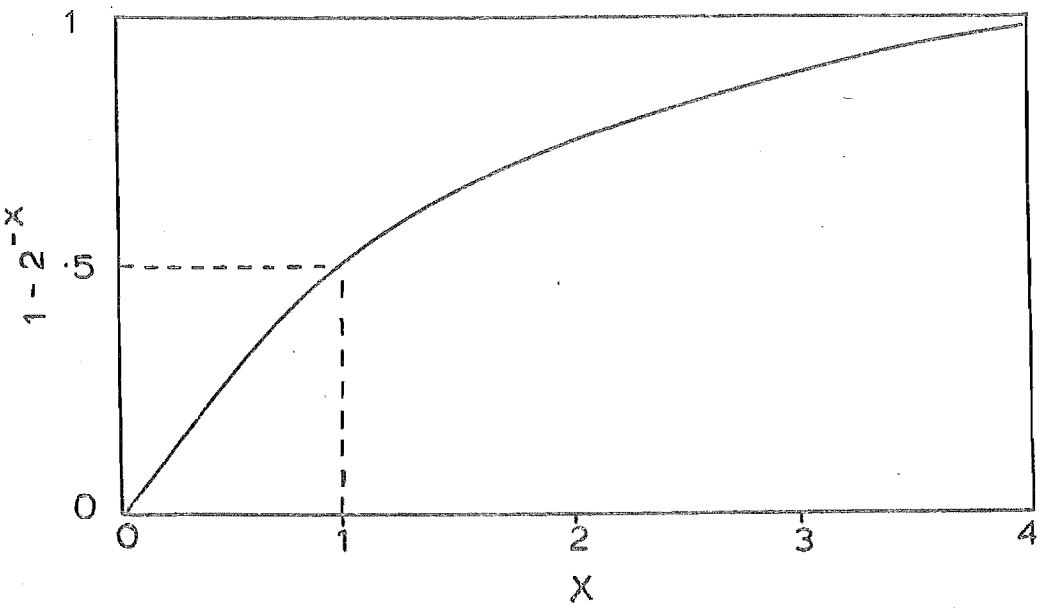


Fig. 9.1 A graph of the relationship between factor intensity (x) and its influence on yield ($1-2^{-x}$). (After Verduin, 1964).

To determine the relative intensity value for each factor, it is necessary to know the intensities at which $\frac{1}{2}$Y opt. and zero yield occur when all other factors are optimal.

In this study these values can be estimated from the growth response to temperature, phosphorus and nitrogen

obtained in the laboratory with zero yield found by extrapolation. The validity of these values can be tested by using them in Verduin's equation to predict yield for factor combinations as tested in the laboratory. The calculation of the relative intensity units for temperature, phosphorus and nitrogen is shown in Table 9.1. The values for phosphorus were determined from experiment II and those for nitrogen from experiment IV.

Table 9.1 Estimated relative intensity of temperature, nitrogen and phosphorus.

Factor		Zero yield	$\frac{1}{2}$Y opt.	Relative intensity
(A)	Temperature			
	Attached plants	6.8°C	8.6°C	1.8°C
	Detached plants	1.0°C	9.0°C	8.0°C
(B)	Nutrients			
	Nitrogen	0 g/m^3	0.03 g/m^3	0.03 g/m^3
	Phosphorus	0 g/m^3	0.1 g/m^3	0.1 g/m^3

Actual yields from experiments VII and VIII on the effect of temperature are compared with the levels predicted from Verduin's equation and are shown in Table 9.2. Y opt. is assumed to be 42% increase in area for the attached plants and 27.5% for the detached plants. The equation tends to over-estimate yield for the attached plants and underestimate it for the detached plants. This may be the result of a poor estimate of zero yield or due to a difference between the nature of the curve obtained in the experiment and that assumed in Verduin's equation.

Table 9.2 Predicted and actual yields in response to temperature.

	^{o}C	Relative intensity		Predicted yield	Actual yield
Attached plants	8.6	1	$(1-2^{-1})x42$	21.0	22.5
	10.4	2	$(1-2^{-2})x42$	31.5	27.0
	12.2	3	$(1-2^{-3})x42$	36.8	29
	14.0	4	$(1-2^{-4})x42$	39.4	34.0
Detached plants	9	1	$(1-2^{-1})x27.5$	13.8v	14
	13	1.5	$(1-2^{-1.5})x27.5$	15.7	20
	17	2	$(1-2^{-2})x27.5$	20.6	25.5

The values for phosphorus and nitrogen determined in experiments II and IV have been tested against the actual yields found for experiment V. The results are shown in Table 9.3.

Table 9.3 Predicted and actual responses to nitrate and phosphate additions.

P	N	Y = Y opt. $(1-2^{-N})$ $(1-2^{-P})$	Actual yield
0.01	0.03	1.1 (8.8)	8.8
0.3	0.03	12.1 (20)	16.5
0.6	0.03	13.6 (22)	27.1
0.01	0.3	1.9 (10.6)	10.6
0.3	0.3	24	18.4
0.6	0.3	27	26.2
0.01	0.6	1.9 (10.6)	18
0.3	0.6	24	22.5
0.6	0.6	27.2	27.5

(8.8) etc. = adjusted predictions

The predicted values do not agree with the actual values, particularly for low P and N combinations and for the 0.6 P 0.03N combination. There appear to be two reasons for these discrepancies. Firstly, the curves obtained in experiment V do not approach zero in the same fashion as the theoretical curve. This relatively high growth at low phosphorus and nitrogen levels could be due to the algae utilising P and N stored in the thallus. The second and more serious difficulty in applying the equation to these data is the occurrence of maximum growth at 0.6 gram per cubic metre P irrespective of the nitrogen concentration until the nitrogen levels exceed 0.06 gram per cubic metre N. The variables are therefore not interdependent at this concentration of phosphorus.

If it is assumed that the growth at 0.01 gram per cubic metre P and 0.03 gram per cubic metre N is mainly due to stored nutrients in the thallus, the predicted values could be adjusted by adding the amount of growth assumed to be the result of stored nutrients to all combinations containing 0.01 gram per cubic metre P or 0.03 gram per cubic metre N. This value is found to be 8.7 by extrapolating the curves to the vertical axis. The adjusted predictions are shown in brackets in Table 9.1. The yields predicted by the adjusted equation are now a reasonable approximation of the actual yields with the exception of combinations where either P or N is optimal.

To test the validity of making the above adjustment, it would be necessary to measure growth of algae which had absolutely no stored nutrients. Waite and Mitchell (1972b) allowed for this by keeping the experimental plants in

0.04 gram per cubic metre P for two weeks and assumed that the plants would utilise the stored phosphorus during this period. Their results, however, follow the same pattern as those obtained in this study which suggests that the algae can grow in very low phosphorus concentrations.

The Verduin equation does not give accurate predictions due to the differences between the theoretical curve and the actual response curves and also as a result of the lack of interdependence of P and N at optimum P values. It may be possible to modify the equation to allow for the difference in the curves but the over-riding effect of optimal P cannot be incorporated into this form of equation.

To apply this form of equation successfully to the field results, it would also be necessary to incorporate functions to express the effect of exposure time, of removal of plants by the currents and of substrate frequency on standing crop levels. There are insufficient data available to judge whether or not factors affecting standing crop independently of the potential productivity will also influence yield in the form of $Y = Y_{opt.} (1-2^{-x})$.

Although Verduin's equation does not fit all of the data from this study, an equation of this type is of more value than the single limiting factor concept in explaining responses to interdependent variables. If the equation could be modified to allow for the dominating influence of optimal phosphorus concentration and if factors such as grazing and substrate availability could be included, it should be possible to develop a model to predict future growth rates and standing crop. The information available suggests that the nutrient levels and temperature would be key factors in any

such model.

5) THE RELEVANCE OF THE RESULTS OF THIS STUDY TO PROPOSED CHANGES ON THE ESTUARY

The Christchurch Drainage Board has proposed the erection of a barrage near the mouth of the Estuary and the deepening and straightening of the river channels by dredging. The aim is to steepen the hydrological gradient in the rivers and to exclude the sea water from the Estuary at high tide, thereby alleviating surface flooding.

The barrage would have the effect of decreasing exposure times, thus favouring the algae by extending the area suitable for their growth and by increasing the active period per tidal cycle. It would also reduce the flushing of the Estuary, resulting in a longer retention period for the sewage effluent which would further increase the nutrient levels in the water. If the barrage were closed only during periods of river flooding, the overall effect may not be significant, however, regular or prolonged closure of the Estuary would create more favourable conditions for the algae. The very dense growths of algae in McCormack's Bay are indicative of future developments on the Estuary if exposure periods decreased or if flushing were reduced.

The effect of dredging the river channels is more difficult to predict as it is not clear what changes would occur in exposure or drainage patterns on the mudflats. It seems likely that the widening of the channels would give faster drainage and decreased exposure times in at least some parts of the mudflats. The result of these changes would depend on the area affected; for example, increased exposure

periods on Brighton Spit would have little effect as the area is already unsuitable for algal growth. The influence on mudflat drainage would also depend on the type of bank left on the channels. If the top of the bank were higher than the surrounding mudflats, then the surface water would remain at low tide producing favourable conditions for the algae.

The net effect of the channel dredging on algal standing crop would also depend on whether algae can settle on the sides of the channels. This may be possible since the sides of the proposed channels would slope at a gradient of 1/20. The proposed bed width of 320 feet is considerably greater than at present and may decrease the current velocities sufficiently to permit algal settlement. If settlement did occur in this area, growth would be considerable due to the favourable exposure regime which may offset any decreases in productivity on the mudflats. The dredging as proposed by the Drainage Board could, therefore, either increase or decrease algal growth, depending on the effect on mudflat drainage and on the settlement of algae on the sides and bed of the channels.

A second scheme has been proposed by the Christchurch City Council which involves deepening the central region of the Estuary to provide deeper water for boating and to retain more water at low tide. The decreased exposure times and the reduced flushing would undoubtedly favour algal growth, especially in the central region which is at present unsuitable for algal growth at present. This would not only be ecologically undesirable but would also detract from the recreational value of the area.

A number of other schemes have also been put forward to

increase the recreational potential of the Estuary, most of which involve dredging and reclamation. These schemes do nothing to improve the water quality which is an essential prerequisite for increased recreational use of the Estuary, and some would tend to increase the retention of pollutants.

6) POSSIBLE METHODS FOR REDUCING ALGAL STANDING CROP

This can be approached in two ways; either by eradicating the algae or by making growth conditions less favourable.

(a) Eradication of the algae

I. Harvesting: The use of heavy machinery, such as bulldozers would be limited by the very soft muds and would also destroy many desirable species of plants and animals. Harvesting from shallow draught boats at high tide would be possible but the murky waters would make it difficult to detect the main concentrations of algae so that attached plants could easily be missed. Any form of mechanical removal would tend to leave behind fragments which could regenerate under suitable conditions.

II. Chemical control: Algicides have been used to achieve temporary control of algae in lakes (Slakekova and Sladeck, 1968; Mackenthum, 1969; Fitzgerald, 1971) but their use could not be recommended in the Estuary. Many of the compounds such as rosin amines, mercury and copper compounds are toxic to fish and other desirable species. In addition, the regular tidal flushing would necessitate high dosage rates to compensate for the dilution by sea water, which would increase the cost and potential danger to the rest of

the biota.

Both harvesting and chemical methods would give only temporary control and would disrupt the rest of the biota. Since the most undesirable feature of large growths of algae is the odour from the rotting drifts trapped above the high tide mark, these areas only could be cleared relatively cheaply with little disruption to the rest of the Estuary but would alleviate, at least temporarily, the "smell problem".

(b) Control of the growth conditions

The only growth factors over which man has any control are the nutrient levels. Since the eutrophic condition of the Estuary originates from domestic or industrial wastes, the elimination of this supply would significantly decrease the nutrients available to the algae. The two main alternatives, piping the sewage out to sea, or tertiary treatment to remove the nutrients before discharge, are discussed by Knox and Kilner (1973). The sea outfall would produce the greater reduction in nutrient supply but may cause pollution of Brighton Beach. Present tertiary treatment methods, on the other hand, would allow only an 80-90% reduction in nutrient levels. The improvement in the nutrient levels in the water under either scheme would depend on the rate of leaching of nutrients stored in the sediments.

Even if all the nutrients from the sewage were removed, sufficient would remain from natural sources to support algal growth, though at a lower rate as indicated by the laboratory experiments. To allow the standing crop to reach nuisance levels, a longer period of favourable conditions would therefore be needed and fewer, if any,

summers would fulfil these requirements.

Nutrient removal by either of the methods discussed above would be considerably more expensive in the short term than either harvesting or chemical control but it is considered to be the only permanent solution. It would also have beneficial effects on the biota as a whole as well as increasing the recreational potential and the aesthetic qualities of the Estuary.

CHAPTER X

CONCLUSIONS

1) TAXONOMY AND LIFE HISTORY

The dominant alga during the period of this study was Ulva lactuca L. which occurred in four forms.

(a) A low growing rosette-shaped "winter" plant, attached to the inside of shells and on the retaining walls.

(b) A large ribbon-like 'spring' plant with fronds up to 30 centimetres long attached to shells or any other firm substrate.

(c) A large expanded sheet-like 'summer' plant up to 1.5 metres across which is unattached and floats freely within the Estuary.

(d) A smaller unattached bullate form resembling Ulva laingii Chapman but which has been shown to be the result of low salinity on aging drift plants of U. lactuca. This form, therefore, appears to be an ecad rather than a separate species.

The detached forms usually make up the majority of the population in summer and under calm conditions may persist throughout the winter and regrow in the following spring. Regeneration in spring may also occur by swarmer production from the prostrate winter form.

The only other alga which was found in significant

quantities was Enteromorpha ramulosa (Smith) Hooker. This was common in both the summers included in this study, occurring in four forms.

(a) Small, sparsely branched 'winter' plants attached to the retaining walls along the shore of the Estuary.

(b) A larger prolifically branched form attached to shells on the mudflats.

(c) A large attached plant similar to (b) but more coarsely branched.

(d) Detached free floating plants with broader branches than the attached forms.

Only the small plants on the retaining wall persisted through winter; regrowth in spring, therefore, was from reproduction of these.

2) ALGAL DISTRIBUTION

The overall distribution pattern is determined by the tidal regime. The long period of exposure excludes the large plants of Ulva and Enteromorpha from the high tide zone and limits their growth in the upper mid-tide zone. The prostrate winter form appears to be more resistent to exposure than the larger plants, but as they occur in shaded areas on the retaining wall, they may be subject to less desiccation than their position in the high tide would suggest. The lack of algae in the low water channels results from the combined effects of low salinities and high current velocities.

Within the mid-tide zone, the attached algae are restricted to areas with stable attachment sites, the most important being the shell beds at sample areas A, B and F.

The detached algae accumulate where current flow is reduced, for instance in depressions on the mudflats and in the back-water bays north of where the Avon and Heathcote Rivers enter the Estuary. The detached plants tend to be concentrated in the western region as the flood tide currents have a greater velocity than the ebb tide currents.

The epibenthic snail Zediloma subrostrata which feeds on Ulva and Enteromorpha is restricted to the centre of the Estuary. Its absence from the western region contributes to the higher standing crop and the persistence of the drift in this locality through winter.

The concentrations of phosphorus and nitrogen in the water increase from the mouth of the Estuary to the western region as a result of the high levels in the sewage effluent and in the river water. If the addition of nitrate and phosphate acts as a stimulus to the growth of U. lactuca in the Estuary as it was shown to do in culture, then productivity should also increase upstream from the mouth of the Estuary. The laboratory results indicate that the optimum concentrations are 0.6 gram per cubic metre N and 0.5 gram per cubic metre P with higher levels of N inhibiting growth. At levels below the optima, N and P interact to give higher responses together than when added separately. The high levels of N and P in the thalli from the western part of the Estuary indicate that these elements are available in excess of the algae's requirements in this region. One or more growth factors of unknown nature are present in more favourable quantities in the estuarine and coastal sea water than in the oceanic water.

Seasonal variation

The period of active growth for U. lactuca is spring, summer and autumn (October to May) while active growth in E. ramulosa occurs in summer, namely between November and April. These seasonal patterns in growth can be correlated with changes in temperature. Laboratory experiments indicate a very marked increase in growth rate of U. lactuca between 8°C and 16°C which corresponds to the temperature changes on the Estuary between August and December. The plants from McCormack's Bay have a slightly higher temperature optimum than those from the Estuary. This may be a physiological adaptation to the warmer water in this area.

Light intensity and photoperiod may also affect the seasonal growth pattern but appear to have a less marked effect than temperature changes.

3) YEAR TO YEAR VARIATION

Variation in algal standing crop from year to year can also be related to temperature, the algae being most abundant in warmer than average years. Enteromorpha appears to be less tolerant to low temperatures than Ulva.

The total standing crop in summer is also influenced by the amount of algae carried over from the previous year. This will depend on the wave action in winter, with calm conditions allowing a larger proportion of the drift population to persist through the winter and contribute to the standing crop of the next summer.

The growth responses of Ulva lactuca cannot be expressed in Verduin's limiting factor equation due to

differences between the theoretical and actual response curves and to the lack of factor interaction at optimum nutrient levels.

4) HUMAN ACTIVITY RELATED TO EXCESSIVE ALGAL GROWTH

Of the factors outlined above, only nutrient levels and exposure times can be significantly influenced by man. Any man-made modification which involves decreases in exposure times will produce a longer period of growth with more favourable conditions for the algae.

The nutrient levels on the Estuary have already increased due to the growing volumes of industrial and domestic effluent. The resulting eutrophication may explain the change from a sparse mixed flora in 1929 to the present over-abundance of Ulva and Enteromorpha. Further increases in sewage discharge will stimulate greater algal growth, especially in the areas towards the mouth of the Estuary which at present have sub-optimal nutrient concentrations. Nutrient levels would also increase if flushing of the Estuary were reduced.

Unless measures are taken to control eutrophication, an excessive algal mass can be expected whenever summer conditions are suitable. As growth increases with nutrient concentrations, nuisance levels of growth will occur more frequently than in the past.

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APPENDIX 1

Maps of algal distribution redrawn from previous studies on the Avon-Heathcote Estuary:

Fig. I	Bruce (1953)
Fig. II	Williams (1959)
Fig. III	Rosenberg (1963)
Fig. IV	Webb (1965)
Fig. V - Fig. IX	Cameron (1969)

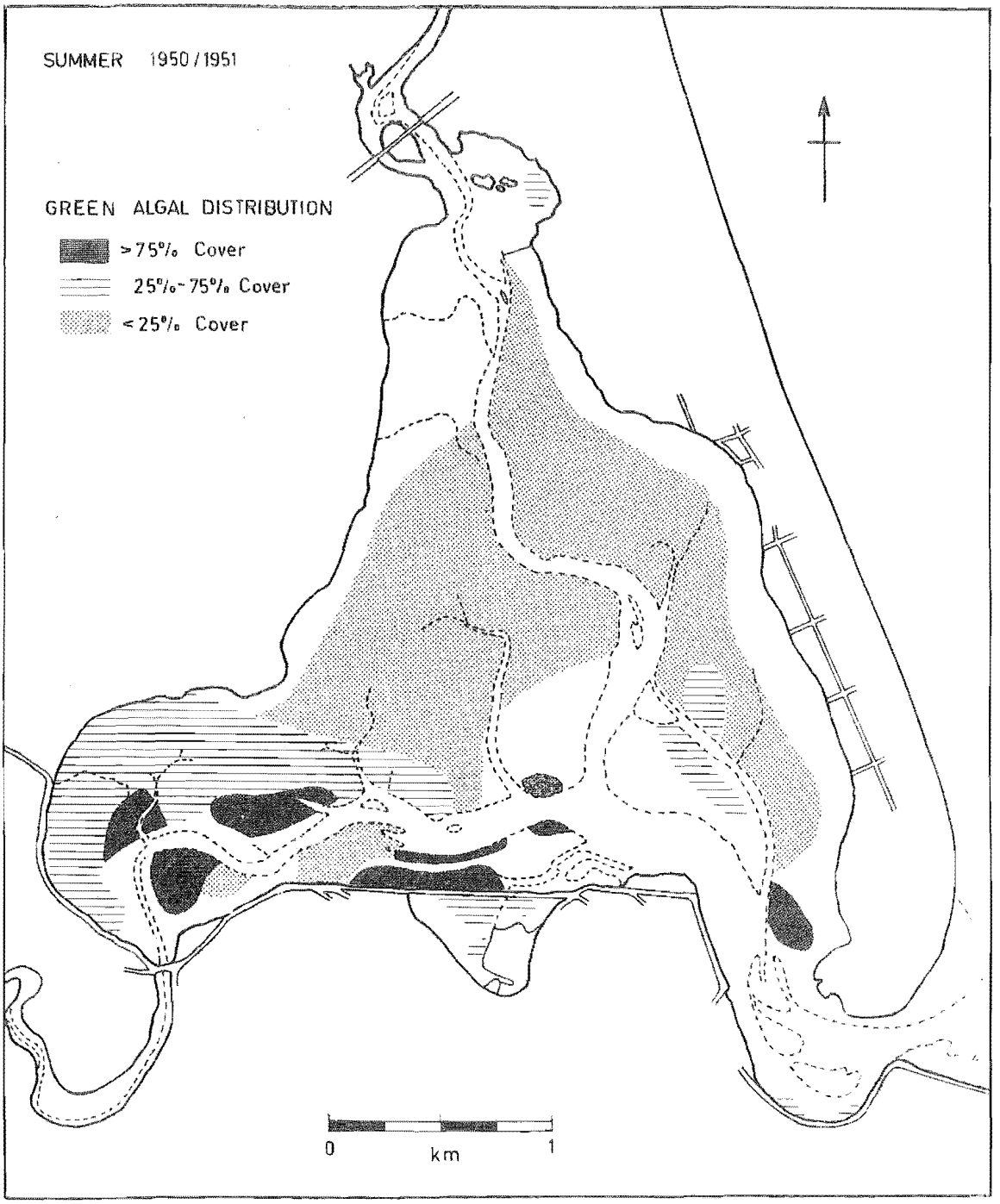

Fig. I Green algal distribution - Summer 1950/1951 (after Bruce, 1953).

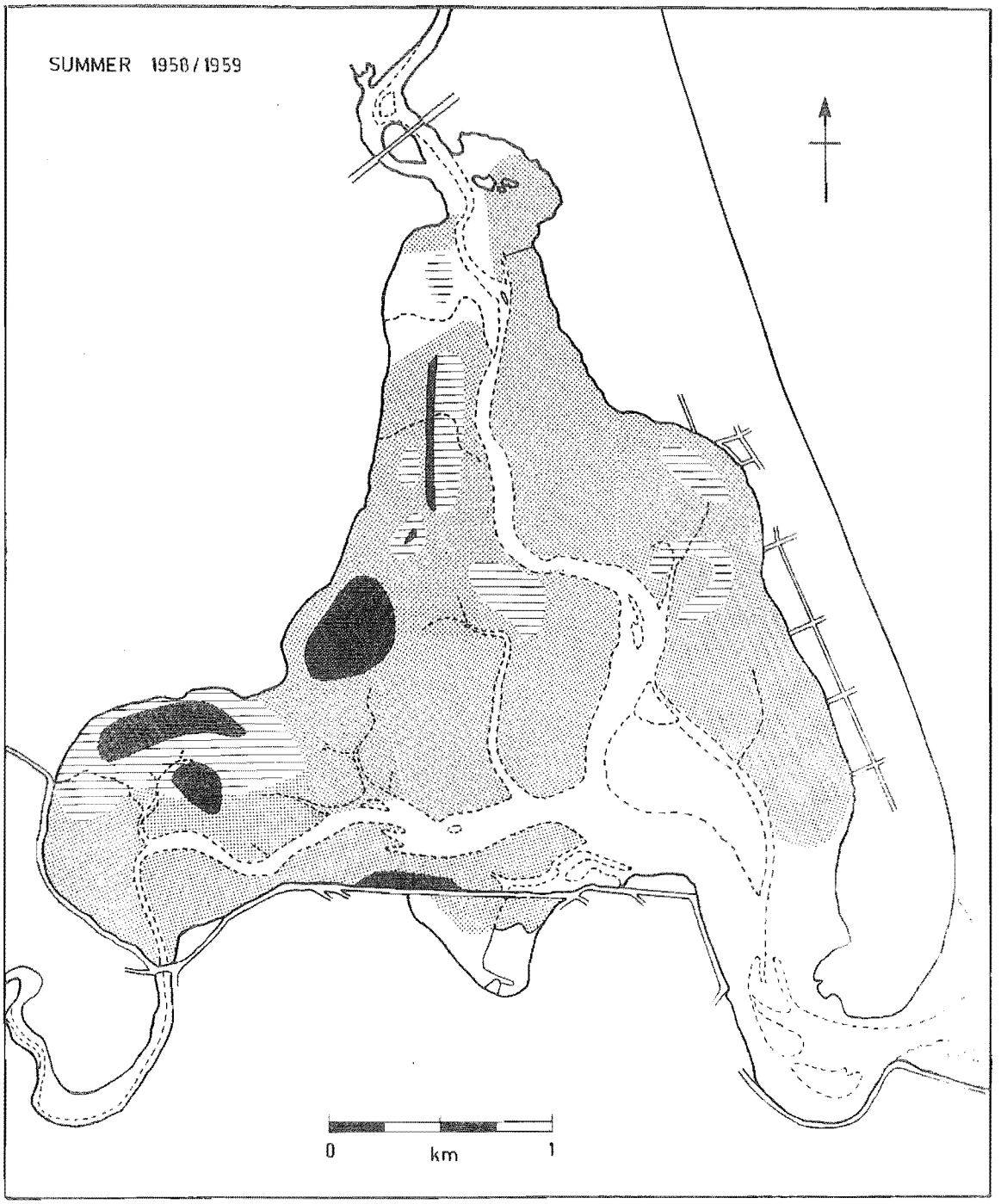

Fig. II Green algal distribution - Summer 1958/1959 (after Williams, 1959): see Fig. I for key.

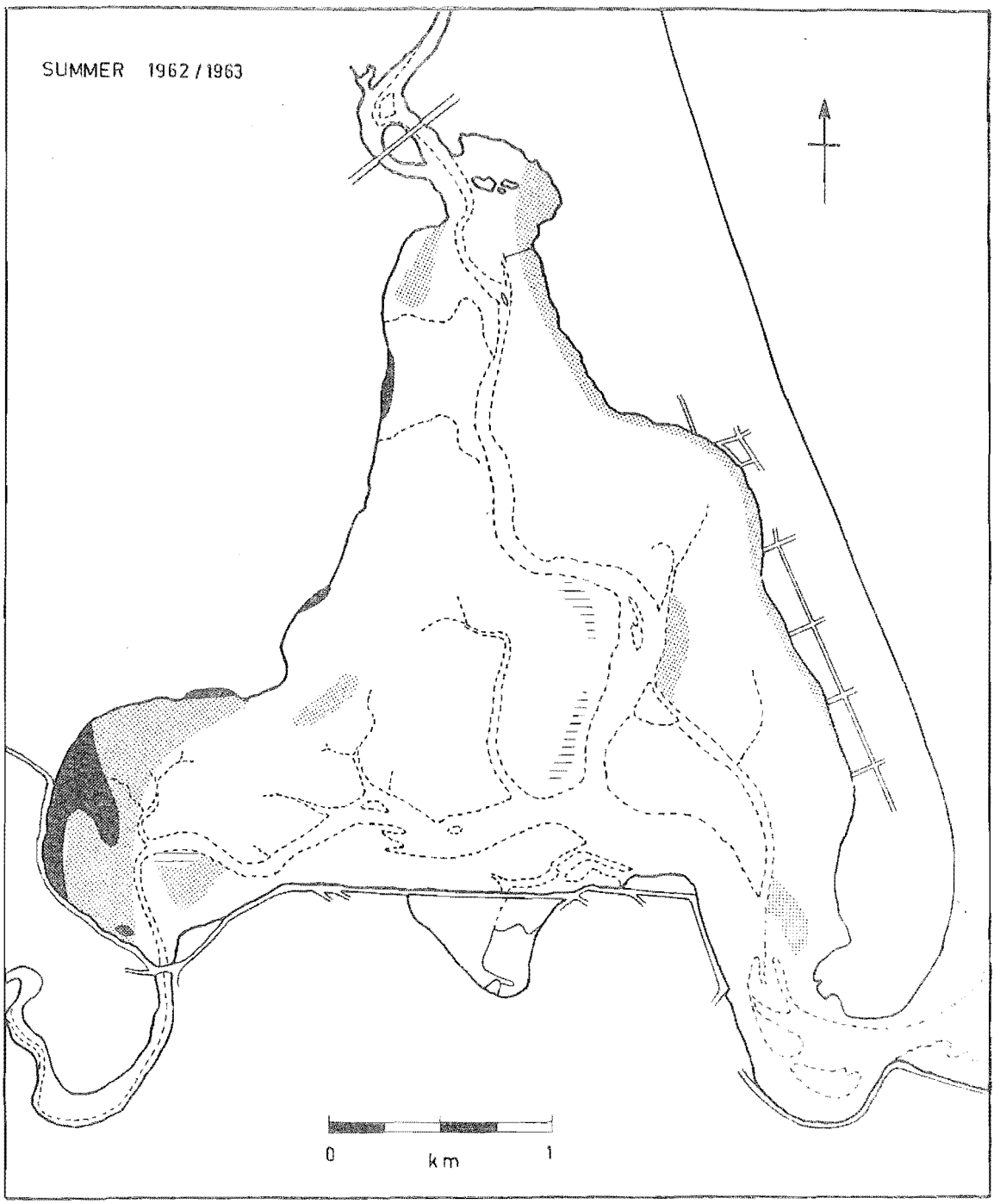

Fig. III Green algal distribution - Summer 1962/1963 (after Rosenberg, 1963): see Fig. I for key.

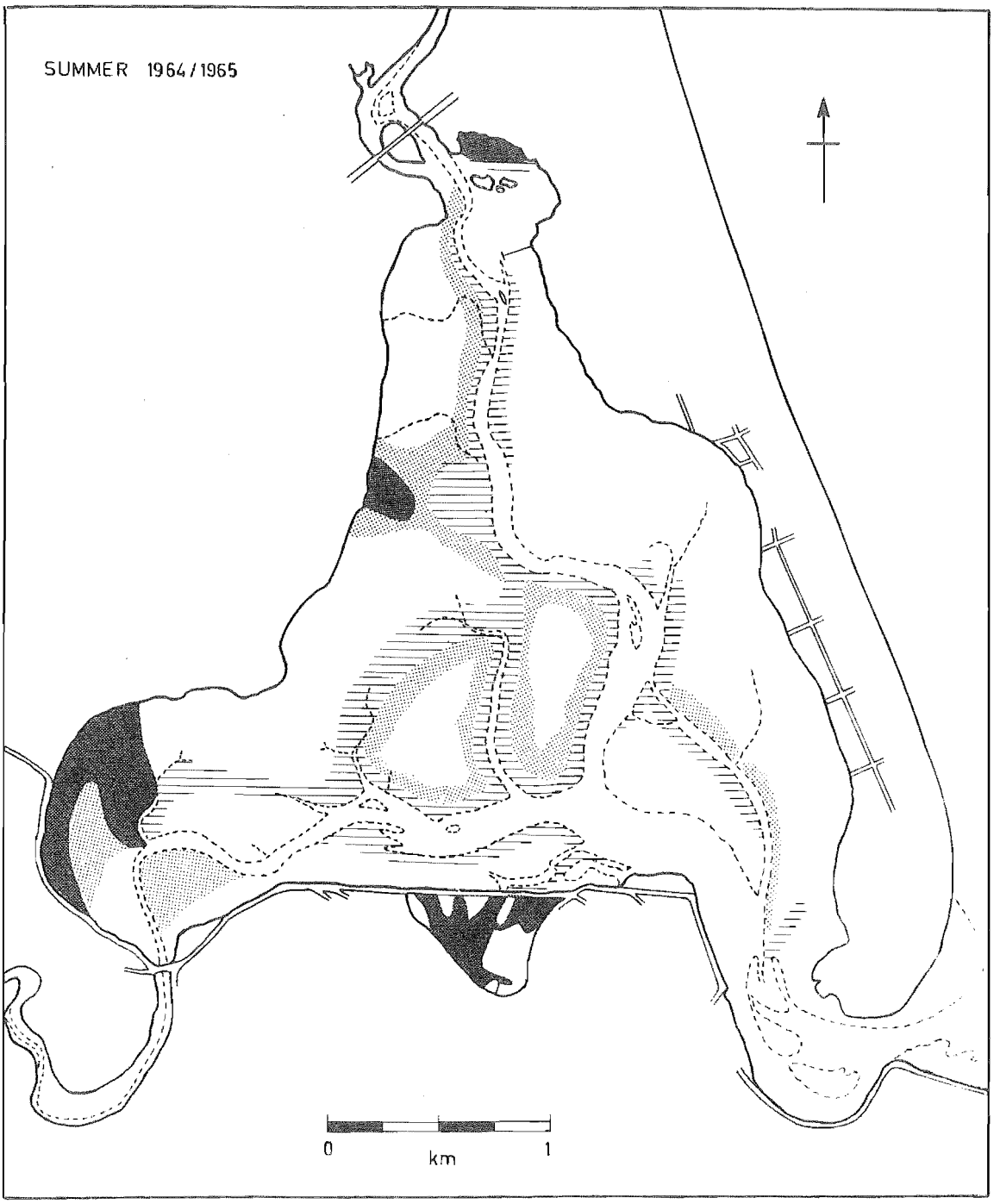

Fig. IV Green algal distribution - Summer 1964/1965 (after Webb, 1965): see Fig. I for key.

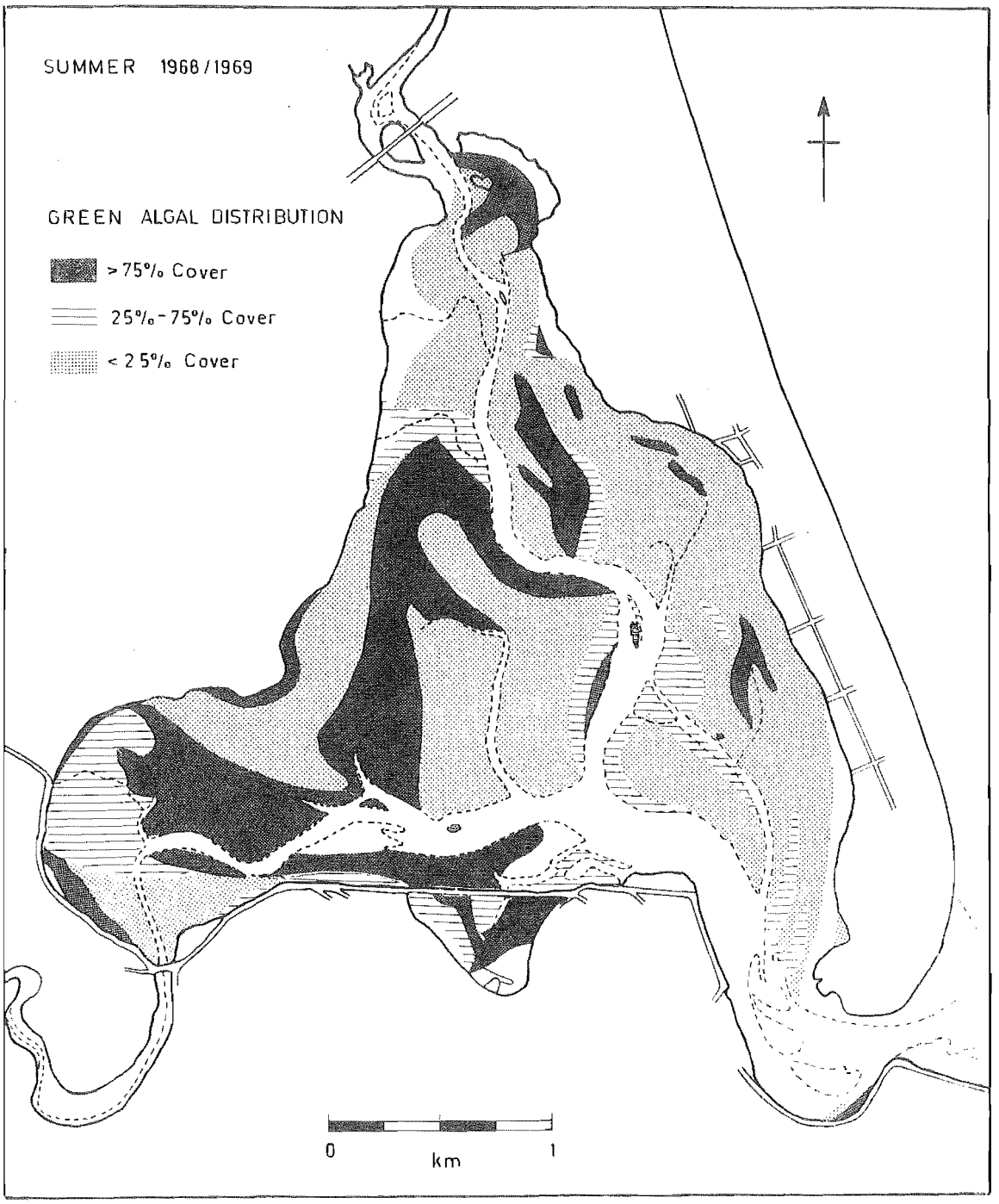

Fig. V Green algal distribution - Summer 1968/1969 (after Cameron, 1970).

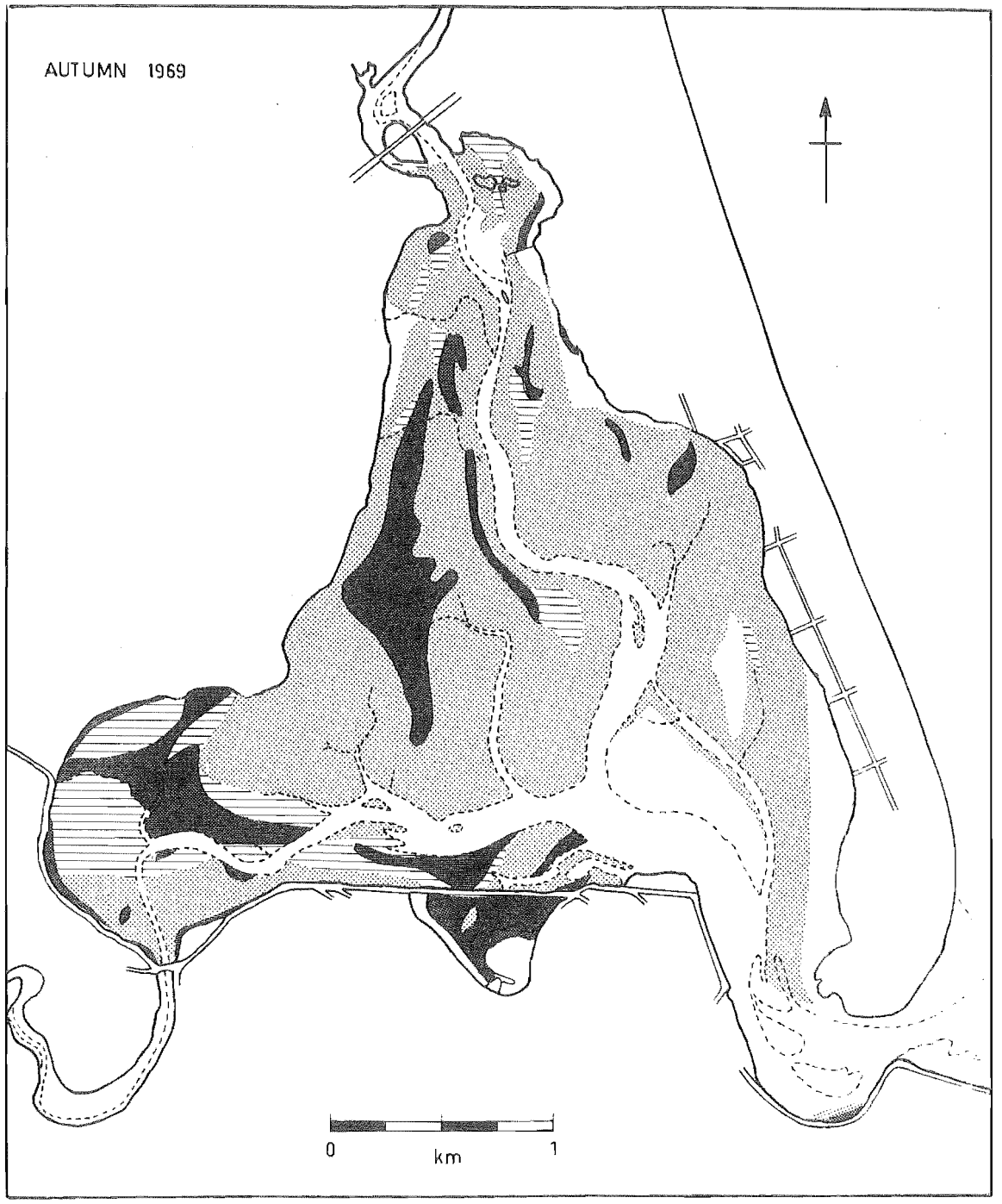

Fig. VI Green algal distribution - Autumn 1969: see Fig. V for key.

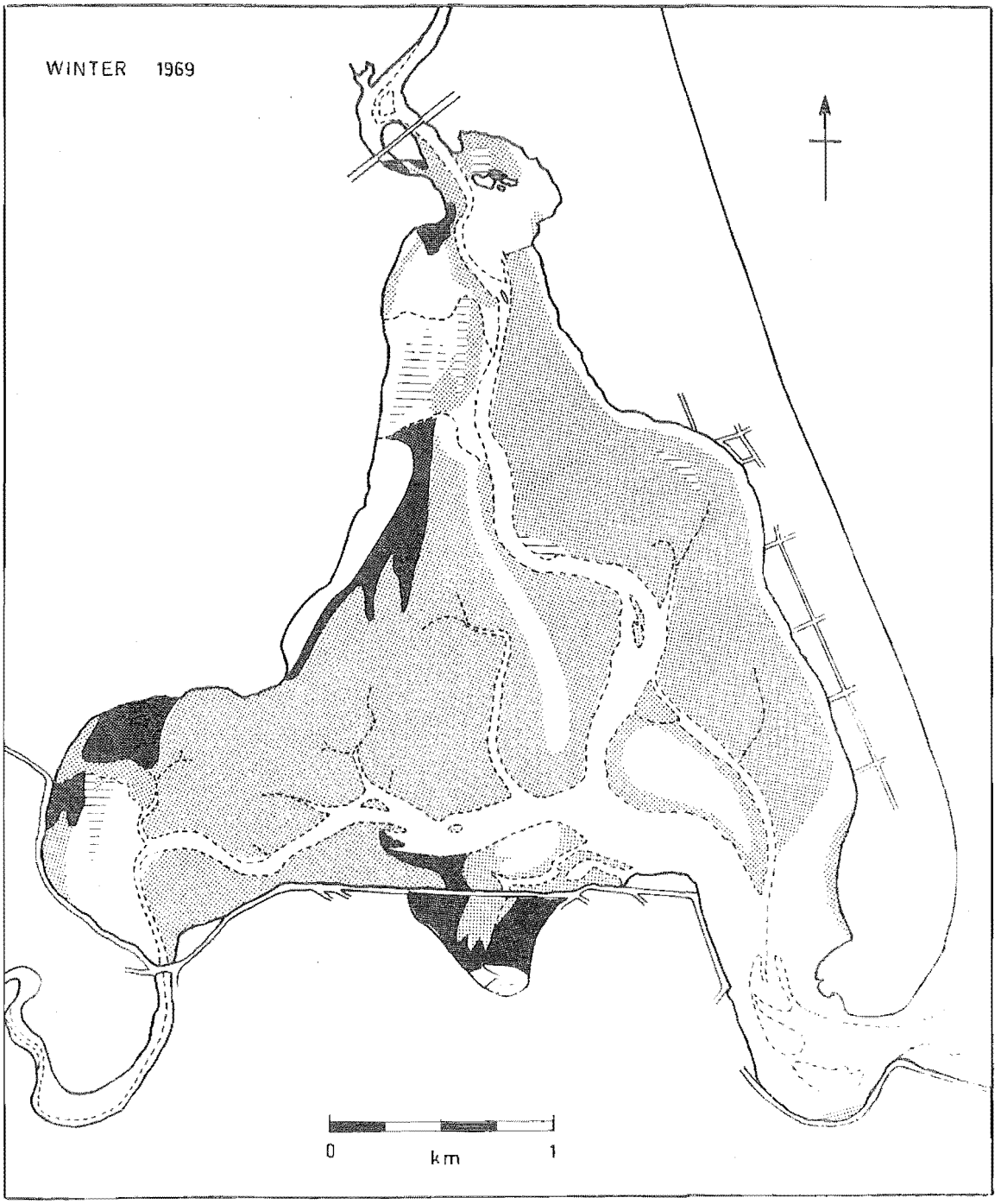

Fig. VII Green algal distribution - Winter 1969: see Fig. V for key.

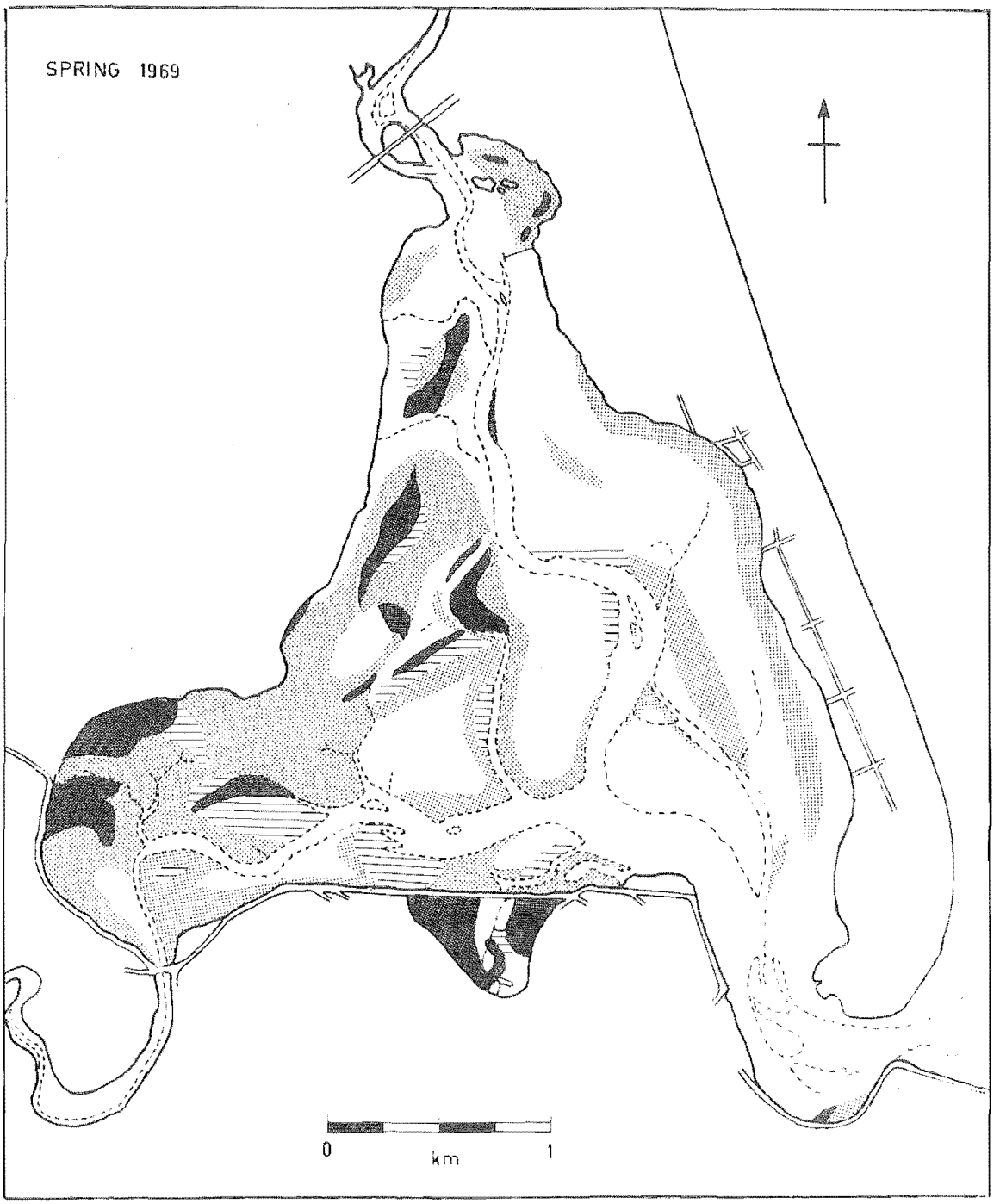

Fig. VIII Green algal distribution - Spring 1969: see Fig. V for key.

Fig. IX Green algal distribution - Summer 1969/1970
see Fig. V for key.

APPENDIX 2

Published work associated with this thesis.

"Distribution of *Euglena obtusa* schmitz and *E. salina* Liebetanz on the Avon-Heathcote Estuary, Christchurch".

D.A. Steffensen.

Mauri Ora, 1974, 2: 85-94.

MAURI ORA, *1974*, *2*: *85- 94*

DISTRIBUTION OF *EUGLENA OBTUSA* SCHMITZ AND *E. SALINA* LIEBETANZ ON THE AVON-HEATHCOTE ESTUARY, CHRISTCHURCH

D.A. STEFFENSEN

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ABSTRACT

Aerial and ground surveys of the Avon-Heathcote Estuary indicated that euglenoid scum was concentrated near the source of effluent discharges, particularly the discharge from the Bromley oxidation ponds. Decreases in numbers of *Euglena* spp. in some regions of the Estuary since 1965 can be related to decreases in the amount of effluent discharged. Distribution up the Heathcote River was limited by a lack of suitable substrate rather than by low salinity, and water content of the mud appears to be less important than formerly suggested. The factor most consistently linked with numbers of *Euglena* was high carbon level in the mud. *E. obtusa* was rendered colourless by treatment with streptomycin, but remained active and at high concentrations in cultures for 3 months. This indicates it is probably heterotrophic, and may explain its occurrence on muds with high carbon content.

INTRODUCTION

Euglena was first recorded in the Avon-Heathcote Estuary by Linzey (1944), who noted "a thin yellow surface scum of some form of *Euglena* near the sewer outlets". The principal component of this scum is a light green, nonflagellated and "highly metabolic" (rapid changes in cell shape) species identified by Bruce (1953) as *Euglena limosa* Gard. This identification was based on morphological and habitat similarities with material described by Gard (1919) and Bracher (1919, 1929) from France and England respectively. Carter (1933) regarded the English material, previously named *E. dese* Ehrenberg by Brancher, as identical with *E. limosa*. Gojdics (1953), however, suggested *E. limosa* may be a synonym for *E. obtusa* Schmitz. This view is supported by G. Leedale (Palmer and Round 1965) who redescribed the English material as *E. obtusa* which is the name I propose to use for the Christchurch material. Williams (1960) recorded a second species, *E. salina* Liebetanz, from the Heathcote River. It is dark green and smaller than *E. obtusa*.

Descriptions of the extent of the scum and concentrations of cells have been included in the following biological surveys of the Estuary: Bruce (1953), Williams (1960), Rosenberg (1963), Webb (1965), Cameron (1968) and Marshall (1971). The present study was carried out in 1969 and 1972, the aim being to monitor any changes in patterns of distribution and to investigate factors affecting these patterns.

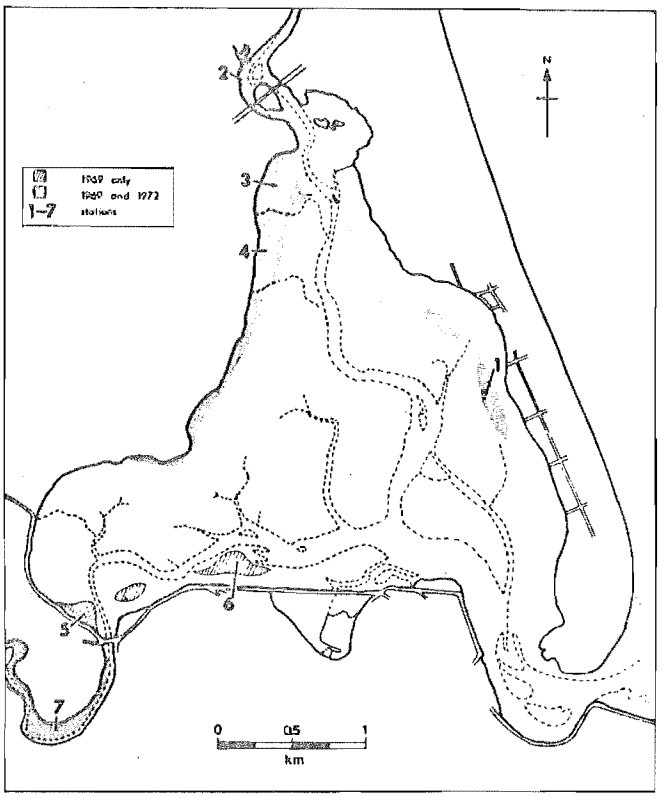

Fig. 1. *Euglena* distribution in the Avon-Heathcote Estuary in 1967 and 1972.

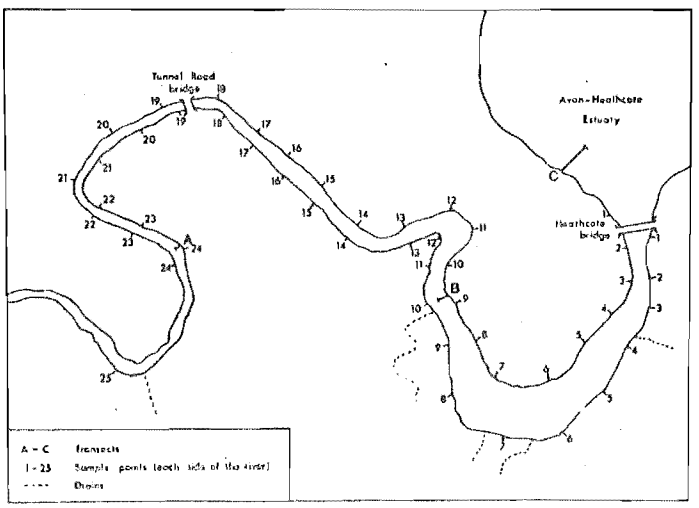

Fig. 2. Lower Heathcote River showing location of 49 sampling points and three transects.

METHODS

The area covered by scum was mapped from a ground survey in 1969 and from aerial colour photographs in 1972 (Fig. 1). Concentration of cells in the scum was sampled at seven stations (see Fig. 1). Numbers of cells were estimated from three surface samples collected on 100 mm^2 pieces of lens tissue at each station. A further three samples, 100 mm^2 and 3 mm deep, were also collected at each station. Preliminary work using frozen core samples had confirmed the observation of Palmer and Round (1965) that all *Euglena* was within 3 mm of the surface at low tide, the time when samples were taken. Each sample was diluted in 200 ml water and three 1 ml aliquots from each were counted in a plankton cell.

In 1969, a series of 24 sampling points were established at mid-tide level at 150 m intervals on each side of the Heathcote River to a point 3.6 km upstream from the Heathcote bridge (Fig. 2). A further sample point was established 4.2 m upstream from the bridge on the southern bank. These points correspond to the limits of firm mud banks. Three high- to low-tidal transects were established at Stations A, B, and C (see Fig. 2). At each sample point and transect level *Euglena* density was determined as above, and three 100 ml mud samples were taken and analysed for water and carbon content. Water content was estimated from weight loss after drying at 110°C for 48 hours. Carbon content was determined from weight loss after combustion at 600°C for 15 hours. Some samples were also analysed by the Schollenberg-Allison technique (Metson 1956) in which the sample is digested in concentrated H_2SO_4 and potassium dichromate, and titrated against ferrous ammonium sulphate.

Salinity of the overlying water was determined at low water neap with a salinometer.

Laboratory cultures were grown in water of different salinities ranging from seawater to freshwater at 18°C. Cell concentration was determined every second day for a period of two months.

RESULTS AND DISCUSSION

In 1969, scum formed a band along the high-tide mark on the western shore of the Estuary. An area of high concentration was centred on the outfall from the Bromley oxidation ponds and extended up the Avon and Heathcote Rivers. There were also thinner patches on the Brighton Spit and near Mt Pleasant. Densities ranged from 20 000 cells per 100 mm^2 on the Brighton Spit to over 250 000 cells per 100 mm^2 near the sewage outfalls. Approximately half the population was contained in the surface samples (Table 1).

The pattern in 1972 was similar to that found in previous surveys with high density patches occurring near the oxidation ponds. A lower concentration in the area adjacent to Mt Pleasant, St Andrews Hill, and west of the Heathcote River could be linked with removal of domestic sewer discharge from this area (in 1965 and 1966) and closing of a starch factory in 1968, which previously discharged highly organic effluent west of the Heathcote River. Over the rest of the Estuary the concentration had increased since 1953, but appears to have stabilised in

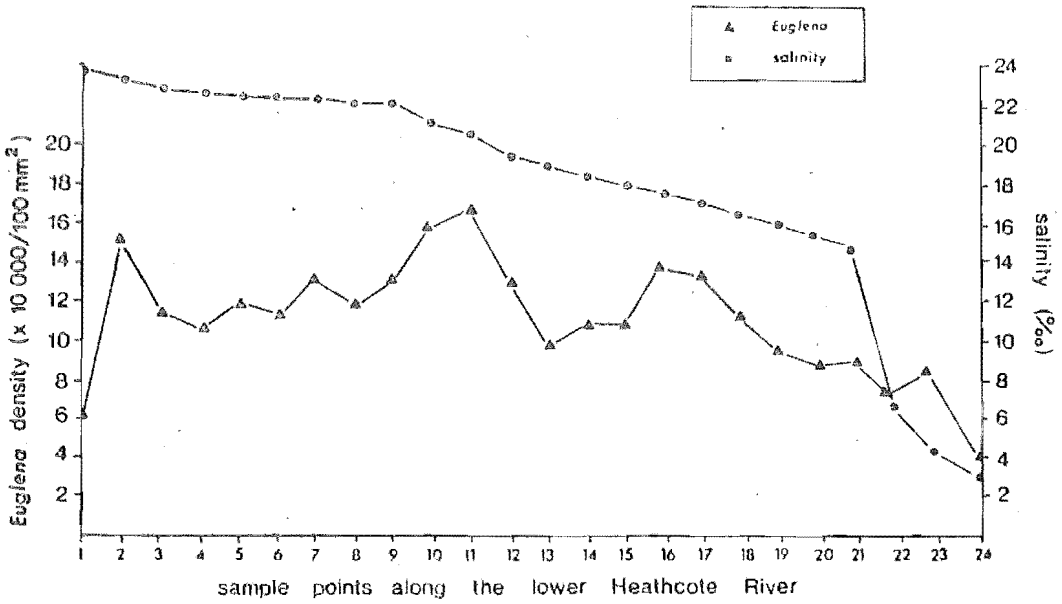

Fig. 3. *Euglena* density and salinity along the lower Heathcote River.

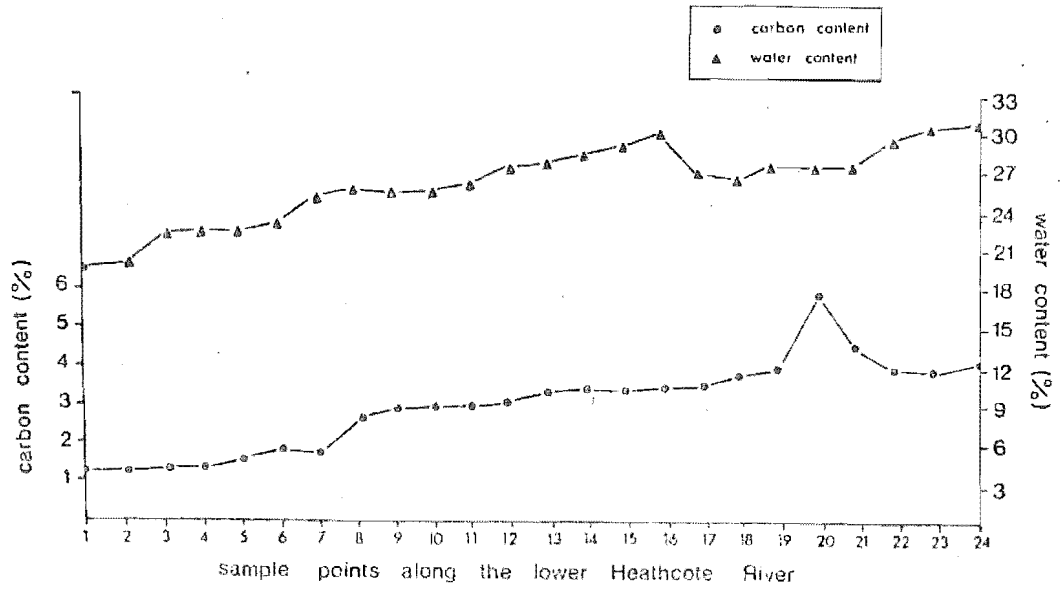

Fig. 4. Carbon and water content of substrate along the lower Heathcote River.

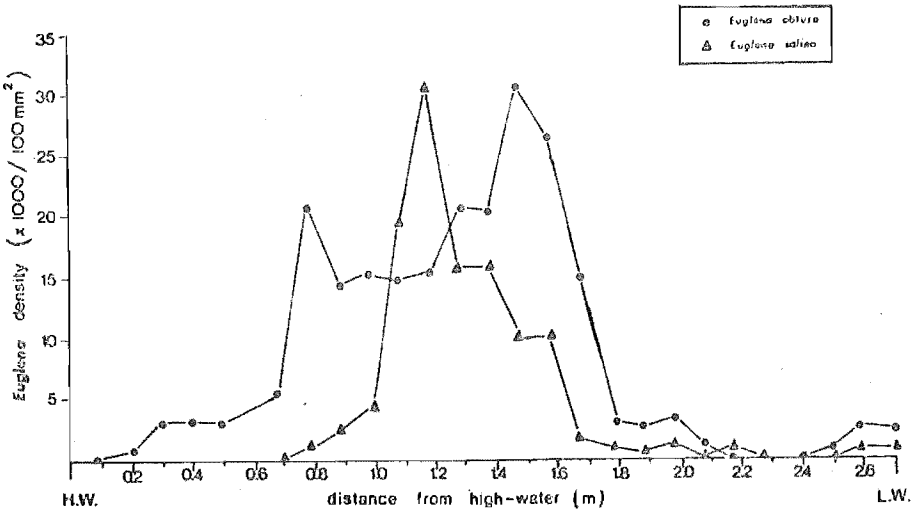

Fig. 5. Density of *Euglena obtusa* and *E. salina* along transect A.

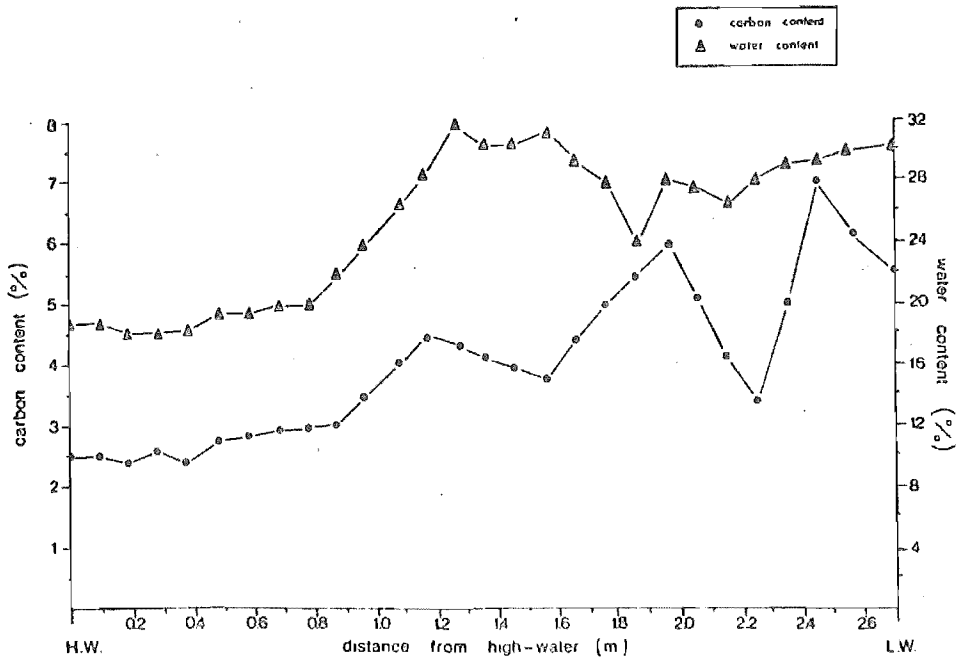

Fig. 6. Carbon and water content of substrate along transect A.

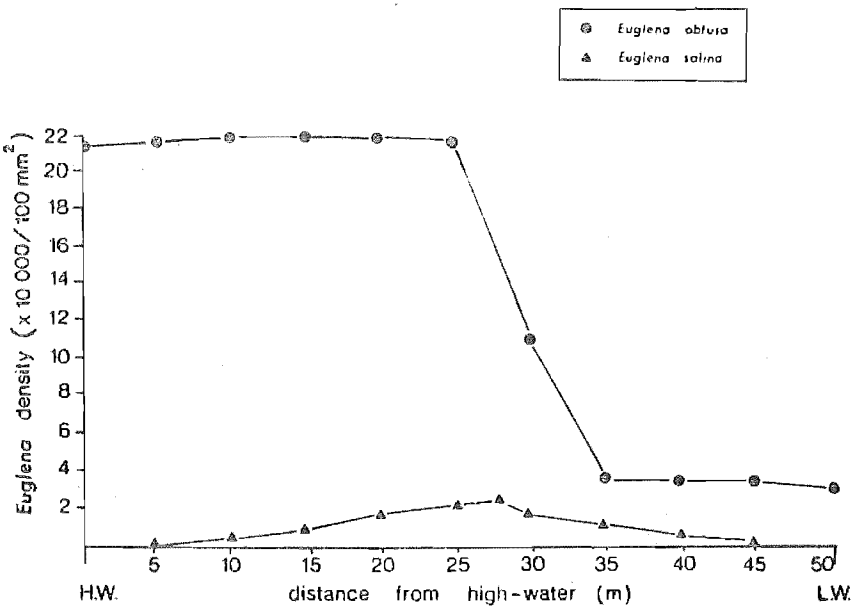

Fig. 7. Density of *Euglena obtusa* and *E. salina* along transect C.

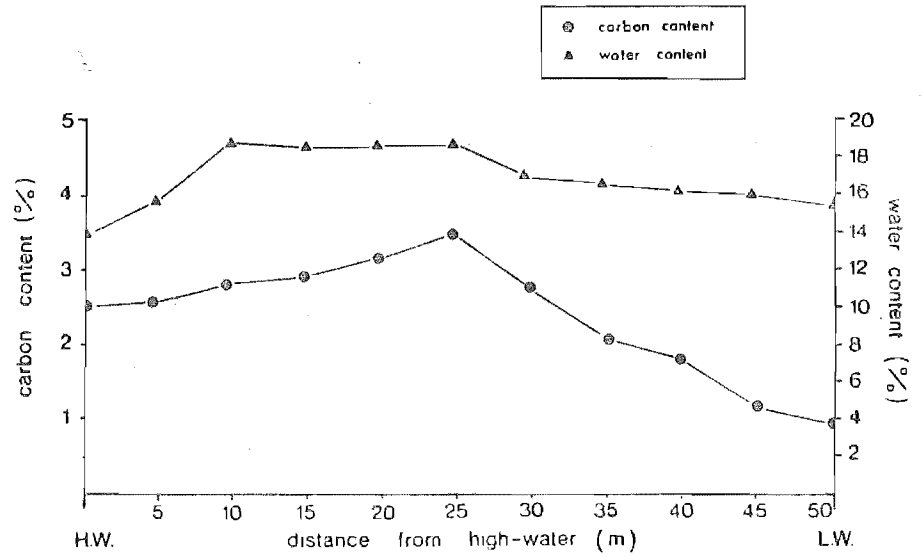

Fig. 8. Carbon and water content of substrate along transect C.

TABLE 1. *Euglena* per 100 m^2 in surface scum samples taken at seven stations in the Avon-Heathcote Estuary.

Station	Numbers at Surface (taken with lens tissue)	Total Numbers (to 3 mm in depth)
I	3 500	10 000
II	130 000	250 000
III	110 000	230 000
IV	153 000	260 000
V	130 000	220 000
VI	76 000	160 000
VII	11 000	20 000

All stations are significantly different from each other at 0.001 confidence level (Least Significant Difference = 7 922) except I and VII (L.S.D. = 6 742; significant at 0.05 confidence level) and II and V (no significant difference).

recent years.

Both species of *Euglena* occurred on the firm, almost flat intertidal mud bank which extends 3.6 km up the Heathcote River from the Heathcote Bridge. Numbers of *E. obtusa* at the sample points (Figs. 3, 4) tended to increase upstream from the Estuary with a maximum of 200 000 cells per 100 mm^2 at sample point 21. Above this point there was a rapid decline in numbers to 40 000 cells per 100 mm^2 at the furthest upstream extension of the bank. An isolated portion of flat intertidal bank a further 600 m upstream (sample point 25) had 23 000 cells per 100 mm^2. *E. salina* was present near the mouth of the river, and at upstream stations, but was absent from samples taken in the middle of the area sampled. Carbon and water content of the mud increased upstream along with *Euglena* numbers, but continued to increase after the latter declined. The decline in *Euglena* numbers corresponded with a sharp decline in salinity of the overlying water. Absence of *Euglena* between 3.6 km and 4.2 km from the Estuary corresponded to the limits of the flat intertidal mud bank.

Along transects A and B highest cell numbers occurred in the mid-tidal region of the mud bank, but numbers decreased rapidly towards high and low watermarks. The decreases at low watermark corresponded to the lower limit of firm mud. Below this was black, highly organic, almost liquid mud which apparently contained very little life of any description. The decline in *Euglena* numbers towards high-tide mark was associated with a sharp decline in carbon and water content (Figs. 5, 6).

Along transect C numbers of *E. obtusa* were very high near high-tide mark (over 200 000 cells per 100 mm^2), but decreased towards low-tide mark. Water and carbon content followed a similar pattern, but substrate remained firm along the full length of the transect (Figs 7, 8). Salinity was 24‰ at all points.

In laboratory cultures *E. obtusa* survived in salinities from 1‰ to 36‰ but higher concentrations were maintained in the 10 to 25‰ salinity range (Fig. 9). *E. salina* did not survive

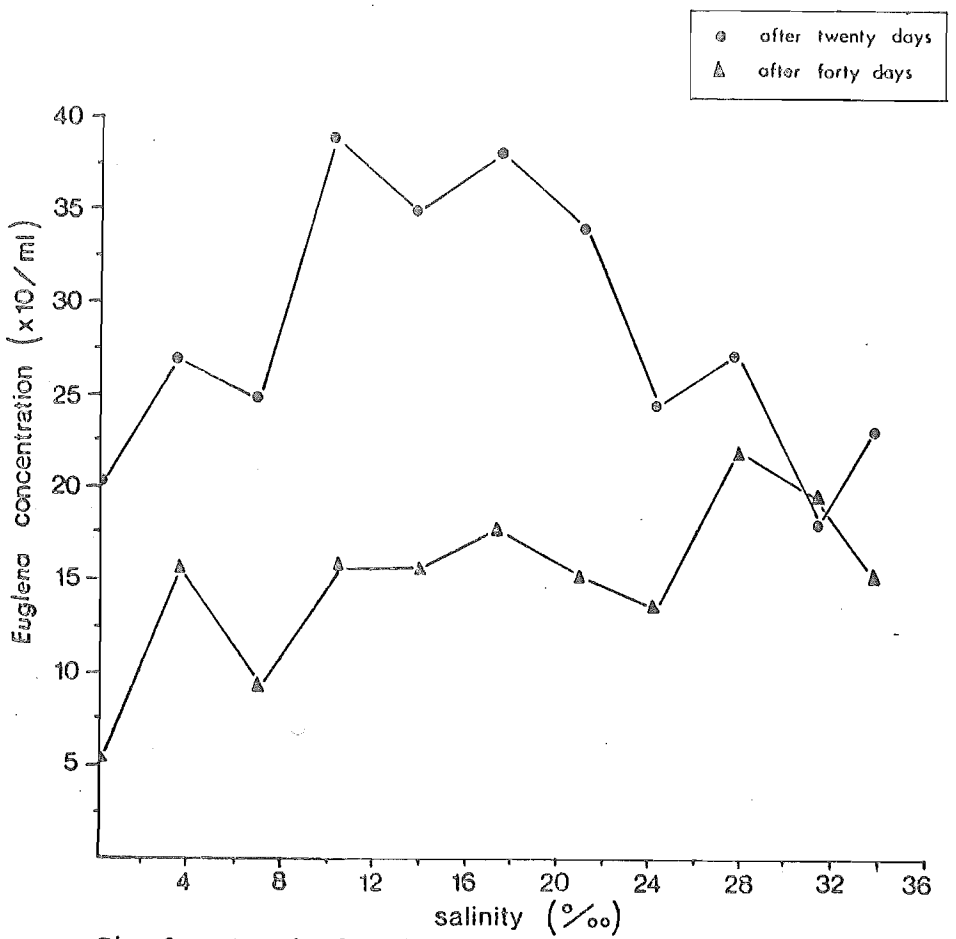

Fig. 9. Growth of *Euglena obtusa* in media of different salinities.

after two days in culture. Cameron (1970) thought that low salinity limited the upstream extension of *Euglena* in the Heathcote River. However, the results from this study indicate that although *E. obtusa* reached its highest density at intermediate salinities, it can survive in culture and in nature at levels very close to freshwater. The upstream limit of *E. obtusa* appears to be determined by the limits of the intertidal mud bank rather than by salinity.

The relationship between the water content of mud and *Euglena* numbers has been studied by other workers. Bracker (1929) found maximum numbers of *E. limosa* at 89% water content but only half the numbers at 82%. Bruce (1953) stated that maximum numbers occurred at 70-80% water content. These figures are 2-3 times higher than those found during the present study in which densities of 200 000 cells per 100 mm^2 were found on muds with only 14% water content. Water content therefore appears to be less important than previously assumed.

The factor most consistently linked with high *Euglena* numbers is high carbon content of the mud and in the Estuary most areas of high carbon concentration are found close to sewage effluent discharge points.

There are, however, a number of other factors which change along with carbon levels in the mud. Bruce (1953) noted that nitrogen levels in the mud followed the organic matter levels and were related to *Euglena* densities on the Estuary. Cameron (1970) found a similar relationship in the Lower Heathcote River. Phosphorus levels tend to follow the nitrogen and carbon levels (Knox and Kilner 1973). When the Estuary is compared with areas in the Lower Heathcote River, however, there is a considerable variation in nitrogen levels associated with very dense areas of *Euglena*. Cameron (1970) recorded 150 000 cells/ 100 mm^2 on muds containing 32 mg N/g. Bruce (1953) found a maximum of 100 000 cells/100 mm^2 on muds containing 750 mg N/g and very low concentrations of *Euglena* on muds with 280 mg N/g. Knox and Kilner (1973) quote levels of 250-500 mg N and over 500 mg P per g sediment in the *Euglena* areas on the Estuary while areas having low concentrations of *Euglena* had over 60 mg N and over 300 mg P per g. The interstitial water, which is the immediate environment of the *Euglena* has a more variable N level, but follows the same pattern as the substrate. Interstitial water in dense *Euglena* areas had nitrogen values ranging from 25 to 150 g/m^2 (Knox and Kilner 1973). It appears that most of the sediments in the Estuary have nutrient levels considerably higher than those associated with very dense *Euglena* populations on the Lower Heathcote River. Even these values are well in excess of normal plant requirements, and I, therefore, suggest that *Euglena* is not responding to high nutrient levels associated with carbon, but rather to the carbon itself. A possible explanation for this response to carbon is that *E. obtusa* feeds heterotrophically, thus utilising organic carbon in the mud directly. Heterotrophy, both obligate and facultative, has been recorded in a number of *Euglena* species (Leedale 1967), and Provasoli (1948) has described viable races of *Euglena* artificially rendered colourless by treatment with streptomycin. *E. obtusa* from the Estuary lost all colour within three weeks in a medium containing 0.6 mg streptomycin/litre, and these cultures remained colourless and at high densities for three months. This indicates an ability to use organic carbon sources in the absence of photosynthesis, which, if it occurs in nature, may explain its association with highly organic muds.

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